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3973 ① Carbohydrate metabolism ② Blood sugar ③ lipid metabolism ④ Protein Metabolism ⑤ Nucleic acid metabolism ⑥ Haemoglobin metabolism ⑦ porphyrins ⑧ Calcium metabolism ⑨ Inorganic phosphate metabolism

phosphatase activity ㉑ Hepatic function

⑪ Iodine metabolism

⑫ water balance ㉒ Renal function

⑭ Acid base balance ㉓ Cerebro-spinal fluid

⑮ Respiratory exchange

⑯ Basal metabolism

⑰ hormones ⑱ vitamins ⑲

⑲ Gastric function ⑳ Pancreatic function

2 term

CLINICAL
BIOCHEMISTRY

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TO THE MEMORY OF

Albert P. Brubaker

Preface to the Fifth Edition

Twenty-five years have elapsed since the authors undertook the preparation of the manuscript of the first edition of this book. At that time there was a lamentably wide gap between the literature of so-called "fundamental" biochemistry and physiology and that of clinical medicine. The majority of students, undergraduate and graduate, particularly practicing physicians, had great difficulty in bridging this gap and, consequently, in taking full advantage of current contributions to knowledge in the basic medical sciences.

The originally stated purpose of this book was to aid in making these correlations. This remains its purpose. However, the gap has narrowed considerably during the past twenty-five years. Clinicians generally have come to realize the truth of the statement by Claude Bernard, almost a century ago, that medicine does not end in the hospital, but rather begins there; that problems raised in the clinic should be passed to the laboratory for investigation and elucidation. Appreciation of this fact has led to closer integration of clinical medicine with the basic medical sciences, and a generation of clinicians has grown up whose routine reading matter is in the language of modern science.

It is possible now to discuss problems of acid-base balance and intermediary metabolism in relation to clinical disorders without much danger of employing terms and concepts incomprehensible to the majority of practicing physicians. In our experience, treatment of these subjects at the elementary level required twenty-five years ago is no longer necessary or desirable. For this reason, normal aspects of metabolism and biochemical mechanisms are discussed here more extensively than previously. It is hoped that this will provide a better basis for an understanding of aberrations of these processes in disease.

The text of the present edition has been virtually completely rewritten. Every chapter has been revised, and much new material has been added, particularly on the following topics: liver function; kidney function; plasma protein abnormalities; biological significance of nucleic acids; uric acid metabolism; porphyrin metabolism; biochemical aspects of diet; iodine metabolism; lipoproteins; fatty liver; potassium metabolism; acid-base balance; endocrine functional diagnosis, especially thyroid and adrenal.

As in previous editions, no attempt has been made to include material that has no relevance to biochemical approaches to the diagnosis or management of clinical disorders. This applies, for example, to most of the vitamins. One feature of previous editions has been eliminated; namely, the separate listings of biochemical abnormalities in various disease states. These were omitted mainly because the index provides this information in essentially the same form. Brief lists of selected reading references have been substituted for the previously longer bibliographies, and direct references have been omitted from the text. It is felt that the purposes of this book are served better by indicating authoritative reviews and monographic discussions of various important topics rather than a large number of original contributions.

As formerly, an attempt has been made to present controversial subjects in an impartial manner, supplemented wherever possible by an expression of personal opinion. There are few statements regarding the clinical significance of biochemical findings that are not supported by personal experience. Thanks are due to many friends and associates for helpful suggestions and advice, and to the publishers for their unfailing cooperation.

Jefferson Medical College, A. C.
Philadelphia M. T.

Preface to the First Edition

Modern advances in physiology and biochemistry have developed a need, not for another laboratory manual, but for a book designed to correlate established facts with problems encountered daily in internal medicine. The rapidity and magnitude of these developments have resulted in the growth of a highly specialized branch of laboratory medicine, namely, chemical pathology. The evolution of this specialty within a specialty has unfortunately tended to remove the clinician still further from a thorough understanding of those phases of internal medicine that require the assistance of the biochemical laboratory for their complete solution.

The remarkably fruitful researches of recent years in the fields of experimental physiology and pathology, by demonstrating the significance of biochemical observations in a constantly increasing number of abnormal states, have correspondingly increased their value to clinical medicine and surgery as well as to the laboratory. Modern practice demands the application of present knowledge regarding aberrations of endocrine, renal and hepatic function, abnormalities of organic and inorganic metabolism, nutritional defects, edema, dehydration, etc., in all branches of medicine as well as in pre- and postoperative treatment. The enormous increase in the use of chemicals in industry and in the treatment of disease and the growing appreciation of the possibly deleterious effects of such agents upon the organism have also increased the service that the biochemical laboratory may render to the clinician. The intelligent employment of these facilities will be of fundamental value in the increasingly important field of industrial toxicology.

The essential function of the laboratory is to supply the clinician with information which will complement that which he may obtain by other methods. Each patient presents a problem which cannot possibly be appreciated on the basis of dissociated laboratory studies. However, in order to take full advantage of the findings of the biochemist, the clinician must have a clear understanding of the significance and limitations of the results of laboratory investigation. This must be based upon an appreciation of the biochemical and physiologic factors involved in the preservation and alteration of organ and tissue function, for it may be stated, more truly than ever before, that physiology is the handmaid of medicine.

The progress made in the fields of biochemistry, metabolism, nutrition, colloidal and physical chemistry is based largely upon observations of a highly specialized and technical nature. This often renders the original literature unavailable to the majority of students and clinicians. As a result they usually either accept the brief interpretative statements made in most works on diagnosis by laboratory methods or they rely upon the chemical pathologist for an interpretation of his findings. The position of the latter is little better than that of the clinician who is required to explain the significance of an enlarged liver in an individual whom he has not seen and concerning whose clinical history and physical condition he knows nothing. Experience in the laboratory and in the clinic has impressed the authors with the difficulty which students and physicians experience in bridging the wide gap between abstract biochemistry or physiology and clinical medicine. Books and articles in abundance have been written for and by specialists, but only a few have attempted to interpret specialized knowledge for the physician. The undergraduate student of medicine and the progressive physician wish to be familiar with the applications of biochemistry to clinical medicine and surgery. They should be as well acquainted with the limitations as with the significance of biochemical findings in any given case. This volume constitutes an attempt to supply this information.

Haldane has stated that the aim of physiology is to consider how the internal environment of the body is kept constant in spite of continual alterations in the external environment. The aim of this treatise is to consider how the internal environment of the body is altered by certain specific changes in tissue and organ physiology. It is further intended to indicate the manner in which the physician may best avail himself of information which can be obtained by biochemical studies. To this end the subject of functional diagnosis by chemical methods has been considered in considerable detail. With few exceptions, the technic of laboratory methods has not been discussed, being available in many admirable standard texts on that subject. The discussion has been restricted to those phases of biochemistry which are concerned with problems commonly encountered in clinical medicine and, therefore, purely abstract and theoretical considerations have been excluded.

A. C.
M. T.

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CHAPTER I

Carbohydrate Metabolism

The chief function of carbohydrate in the animal organism is that of a fuel, the degradation of which to carbon dioxide and water represents a major source of energy. In addition, certain products of carbohydrate metabolism aid in the breakdown of many foodstuffs, acting as catalysts or promoters of oxidations. When the supply of these carbohydrate metabolites runs low, owing to faulty metabolism, widespread disorders prevail, as in diabetes mellitus. Carbohydrate can also be used as a starting material for the biological synthesis of other types of compounds in the body, such as fatty acids and certain amino acids. A final and more obscure function of carbohydrate is its role in the structure of certain biologically important compounds, such as glycolipids, glycoproteins, heparin, nucleic acids and other substances.

DIGESTION AND ABSORPTION

Ingested disaccharides and polysaccharides are converted into monosaccharides by the action of enzymes present in the salivary, pancreatic (amylase) and intestinal (invertase, maltase, lactase) secretions. Glucose (dextrose) is the most important of these end products, fructose and galactose being formed in smaller amounts under ordinary circumstances in adults (from sucrose and lactose respectively). These monosaccharides are absorbed from the intestine and carried to the liver in the portal circulation. The processes of digestion and absorption occur gradually, the quantity of glucose absorbed being about 1 gm. per kilogram of body weight per hour following a carbohydrate meal.

Glucose is not absorbed in appreciable amounts from the stomach. Relatively small amounts are absorbed from the colon, particularly under conditions of normal blood sugar concentration. The rate of absorption decreases progressively as glucose passes through the jejunum and ileum. It also appears that the rate of absorption of glucose from the small intestine remains quite constant from hour to hour regardless, within wide limits, of the amount of sugar present and the concentration in which it is introduced. The intestinal mucosa exhibits a highly selective action with regard to the absorption of various sugars, which is independent of the size of their molecules.

The comparative rates of absorption of the monosaccharides may be indicated as follows: galactose (110) > glucose (100) > fructose (43) > mannose (19) > pentoses (15–9). These striking differences, in conjunction with the fact that they are all absorbed from the peritoneal space at the same low rate, i.e., that of pentoses, suggest that at least galactose, glucose and fructose are transferred across the intestinal mucosa by some active process, and not entirely by diffusion.

Moreover, the rate of absorption of a hexose is modified by simultaneous administration of another sugar; e.g., that of galactose is diminished when it is given with glucose. Two mechanisms are apparently involved: (1) simple diffusion, dependent on the sugar concentration gradients between the intestinal lumen, mucosal cells and blood plasma; (2) an active process, probably involving phosphorylation in the intestinal epithelial cells. According to this concept, glucose, diffusing into these cells from the intestinal lumen, is transformed rapidly to glucose-6-phosphate (glucokinase + adenosinetriphosphate (ATP)), thus facilitating the entrance of additional glucose by diffusion, inasmuch as the concentration gradient of free sugar between the lumen and the interior of the cell is thereby increased. Glucose-6-phosphate is promptly hydrolyzed (nonspecific phosphatase) and the sugar passes into the interstitial fluid and portal capillaries (and thoracic lymph) in its original form. This active process involves expenditure of energy (from ATP). Fructose probably undergoes similar phosphorylation (fructokinase + ATP) to fructose-6-phosphate, which is converted to glucose-6-phosphate, with which it is in equilibrium. By this reaction, a portion of the fructose may be transformed to glucose in the intestinal wall. However, this transformation occurs much more actively in the liver.

Phosphorylation of galactose also is assumed to occur (galactokinase + ATP). Whether other monosaccharides undergo a similar transformation in the intestinal wall is not clear; pentoses are apparently absorbed solely by diffusion. Absorption of glucose from the intestine is influenced by the general condition of the organism, being interfered with in infections, various intoxications, prolonged undernutrition and vitamin deficiencies, especially of thiamine, pantothenic acid and pyridoxine. The period of contact of the sugar with the absorptive surface is of importance in this connection, the amount absorbed being diminished with increased intestinal motility, as in diarrheal conditions and gastrocolic fistula. Absorption may be decreased in the presence of abnormalities, structural or functional, of the mucous membrane, as in inflammation (enteritis), edema (congestive heart failure) and celiac disease. It is retarded in hypothyroidism and accelerated in hyperthyroidism. These facts must be borne in mind in interpreting results of the oral glucose tolerance test (p. 23). Diminished absorption in adrenal cortical insufficiency is dependent upon the decreased concentration of Na and Cl in the body fluids, not upon a specific influence of the adrenal hormones on the absorptive process.

Fate of Absorbed Carbohydrate. After absorption into the portal blood, carbohydrates must pass through the liver before entering the systemic circulation, a fact of considerable physiological significance. Certain hepatic mechanisms contribute to the withdrawal of carbohydrates from the blood: (1) uptake of hexoses such as fructose and galactose for conversion to glucose by the liver cells; (2) conversion of glucose to glycogen for storage in the liver (glycogenesis); (3) utilization of glucose, by oxidation, for energy production; (4) utilization of glucose for the synthesis of other compounds, such as fatty acids and certain amino acids. Opposed to these mechanisms are others which lead to the release of glucose by the liver to the blood: (1) formation of blood sugar from hexoses other than glucose by the liver; (2) conversion of liver glycogen to blood glucose (glycogenolysis*); (3) synthesis of blood glucose by the liver from noncarbohydrate sources, such as certain amino acids (gluconeogenesis). The amount of glucose reaching the systemic circulation at any instant is consequently the resultant of the operations of these two groups of opposing processes.

Once it is in the systemic circulation, blood glucose becomes available for utilization by the extrahepatic tissues. From what has been said about portal absorption, it is evident that these tissues are presented with carbohydrate which has already been "picked over" by the liver in a selective manner. It is to be expected, therefore, that the physiological state of the liver will have a profound influence on the carbohydrate metabolism of the entire organism, an expectation abundantly fulfilled in practice.

Endogenous Sources of Glucose. Among the minor carbohydrate sources of body glucose may be listed the small quantities of endogenous galactose, mannose and, possibly, pentoses which may be converted to glucose under certain circumstances. The major endogenous carbohydrate source is liver glycogen. The glycogen of muscle is not directly convertible to blood glucose; however, the lactic acid formed during glycolysis of muscle glycogen can be converted to glucose and glycogen in the liver.

Certain noncarbohydrate sources are available to the organism for production of glucose. Although it is generally believed that the fatty acids cannot undergo net conversion to carbohydrate, the glycerol moiety of lipids can serve as a source of glucose (from which it can also be derived). However, amino acids are the major raw material for the synthesis of carbohydrate from noncarbohydrate sources (gluconeogenesis). It has been estimated that over half of the average animal protein is potentially capable of conversion to carbohydrate. Those amino acids which are carbohydrate-formers are called "glucogenic." The major site of gluconeogenesis is the liver.

UTILIZATION OF GLUCOSE

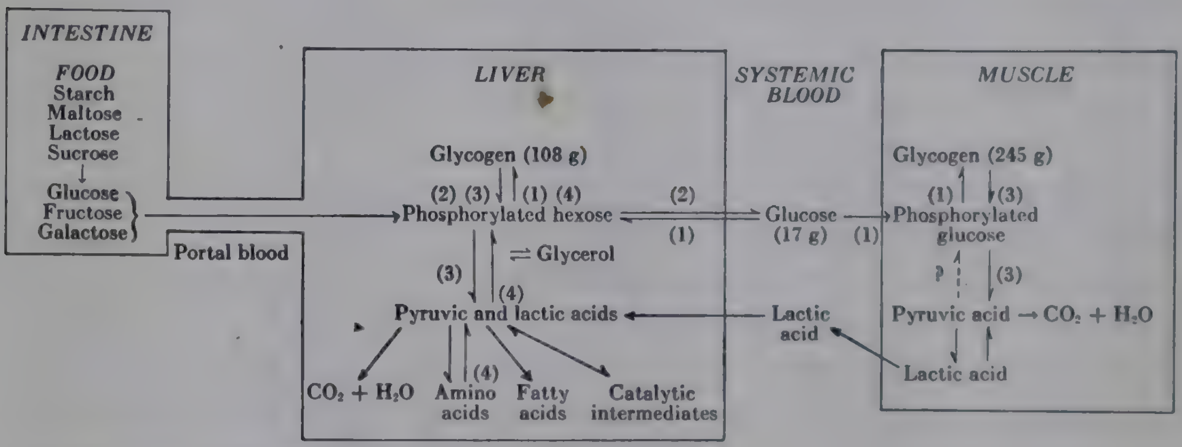
Storage. When a molecule of glucose is introduced into the body or synthesized *de novo* therein, several metabolic pathways are open to it. In the absence of urgent physiological demands for oxidative energy or conversion to special products, excess glucose may be deposited as glycogen in the liver and other tissues (glycogenesis). Inasmuch as the amount of glycogen which can be stored in the body is limited, quantities of glucose in excess of this upper limit are converted to fatty acids and stored as triglycerides in the fat depots. There seems to be no fixed limit to this process, as may be seen from everyday observations on human beings.

Oxidation. In response to physiological demands for energy, glucose may be completely oxidized to CO_2 and H_2O, a process which occurs in all tissues. At certain times, special circumstances in muscle may result in only partial degradation of glucose (glycolysis). The product, lactic acid, is then largely disposed of by other tissues, notably liver.

---

* The term "glycogenolysis" is used by some authors to mean any breakdown of glycogen, whether it leads to blood glucose (one of the two available pathways in liver), or down the glycolytic sequence of reactions to pyruvic and lactic acids (the second of the two pathways of liver, and the probable sole pathway in muscle).

Conversion to Fat. As indicated above, a net conversion of glucose to fatty acids occurs when accommodations for storage as glycogen are exceeded. However, since one of the major metabolites of glucose is in rapid equilibrium with fatty acids (p. 13), a constant interconversion occurs between certain molecules common to the carbohydrate and fatty acid pathways. This interconversion is very rapid, and investigations with isotopes have led to the conclusion that a large proportion of glucose that is oxidized actually forms fatty acids before final degradation to CO_2 and H_2O. It must be noted that the over-all conversion of glucose to fatty acids is irreversible, for reasons which will be pointed out subsequently (p. 8). In contrast, the transformation of glucose to the glycerol moiety of the lipids is readily reversible.


INTESTINE
FOOD
Starch
Maltose
Lactose
Sucrose
Glucose
Fructose
Galactose
Portal blood
LIVER
Glycogen (108 g)
(2) (3)
(1) (4)
Phosphorylated hexose
Glycerol
(3)
(4)
Pyruvic and lactic acids
(4)
$CO_2 + H_2O$
Amino acids
Fatty acids
Catalytic intermediates
(2)
(1)
SYSTEMIC BLOOD
Glucose (17 g)
(1)
Lactic acid
MUSCLE
Glycogen (245 g)
(1)
(3)
Phosphorylated glucose
?
(3)
Pyruvic acid → $CO_2 + H_2O$
Lactic acid

(1) Glycogenesis; (2) Glycogenolysis; (3) Glycolysis; (4) Gluconeogenesis

Fig. 1. Sources and routes of utilization of carbohydrate. (Numerical data for 70 kg. man)

Conversion to other Carbohydrates. Small amounts of glucose are used, directly or indirectly, in the synthesis of certain other carbohydrates which play important roles in the economy of the organism. These include (1) ribose and desoxyribose, required for the synthesis of the nucleic acids (p. 197), (2) mannose, glucosamine and galactosamine, which form parts of the mucopolysaccharides and glycoproteins, (3) glucuronic acid, also involved in the mucopolysaccharides and in "detoxication" reactions, and (4) galactose, which is a component of the glycolipids, as well as of the disaccharide, lactose, secreted in milk (p. 20).

Conversion to Amino Acids. Certain amino acids are not required in the diet, although they occur in the tissue proteins. It may be concluded, therefore, that they are synthesized by the body. Evidence at hand indicates that this group of amino acids, commonly designated "dispensable" or "nonessential" (p. 130), derives its carbon skeletons from glucose or its metabolites. Although all amino acids which are formed from glucose are also "glucogenic," the converse generalization does not hold.

GENERAL PROCESSES IN CARBOHYDRATE METABOLISM

The sequence of catabolic reactions whereby glucose is degraded to CO_2 and H_2O may be divided, for didactic convenience, into anaerobic and aerobic phases. It is currently believed that these phases do not involve separate pathways, but that an initial, anaerobic series of reactions takes place, continuing directly in an aerobic series in the presence of oxygen. The anaerobic phase of glucose metabolism occurs whether oxygen is present or not, its anaerobic character deriving from the fact that participation of oxygen is not required in any of its reactions. "Glycolysis" is the term commonly applied to the production of lactic acid from glucose or glycogen, a phenomenon which is rare under physiological conditions, being largely restricted to circumstances of muscle contraction (p. 11) in which the rate of metabolism of carbohydrate outstrips the oxygen supply to the tissue (relative anaerobiosis).

Under the usual aerobic conditions, lactic acid either is not produced at all, or, if formed, is immediately reoxidized, *in situ,* to pyruvic acid. In any event, the aerobic phase of glucose metabolism begins with the end-products of the anaerobic phase, lactic or pyruvic acids, which, by means of an ingenious cyclic mechanism (p. 7), are degraded stepwise to CO_2 and H_2O. The efficiencies of the anaerobic and aerobic phases are remarkably similar, insofar as recovery of liberated free energy (p. 7) is concerned. However, if calculated in terms of energy obtained per molecule of glucose degraded, the aerobic is superior to the anaerobic by a factor of about 15 to 20. It is interesting to speculate that the energy requirements of the lowly anaerobic organisms, as rather primitive bits of protoplasm, can be satisfied by a process which necessitates the turnover of a large amount of fuel for a small amount of energy, whereas the higher organisms, which have to "get up and go," have had their "burners" converted to more efficient forms.

Anaerobic Metabolism (Glycolysis). Glycolysis can be subdivided into the phases of (*a*) initial phosphorylation, (*b*) glycogen synthesis, (*c*) conversion to trioses (cleavage), (*d*) the oxidative step, and (*e*) formation of lactic acid. These are outlined in Figure 2.

It seems probable that glucose and other hexoses (fructose, galactose) must be phosphorylated for transfer across cell walls or membranes, e.g., during absorption from the intestine and in passing from the blood into the tissue cells. In any event, an initial phosphorylation is an obligatory initial step in the metabolism of glucose (and other sugars). This reaction is catalyzed by a class of enzymes termed "phosphokinases," that concerned with glucose being designated "glucokinase" ("hexokinase"). In the presence of this enzyme and adenosinetriphosphate (ATP), glucose-6-phosphate is formed ("hexokinase reaction"). As indicated in Figure 2, fructose and galactose are brought into the pathway of glucose metabolism at or beyond this point, which means that they are potential sources of blood glucose and liver glycogen.

The "hexokinase reaction" is inhibited by growth hormone (anterior pituitary, p. 16) and adrenocortical hormones (p. 16), this effect being counteracted by insulin (p. 14). These hormones, therefore, exert a fundamental influence upon the first, obligatory step in the metabolism of glucose, which is one reason for the disturbances in carbohydrate metabolism which occur in the presence of abnormal supply of these factors.

Depending on circumstances, the subsequent anaerobic metabolism of glucose may take the direction of (*a*) glycogen formation (glycogenesis) (liver and muscle, mainly) or (*b*) degradation to pyruvic acid and, in muscle, to lactic acid (Fig. 2), with the liberation of energy (p. 11). The reversible reaction, glucose-1-phosphate $\rightleftharpoons$ glycogen, is catalyzed by two enzymes, one of which is phosphorylase, the activity of which is enhanced by epinephrine (p. 17) in liver and muscle, and by the hyperglycemic factor (p. 17) of the pancreas (HGF, glucagon) in the liver.

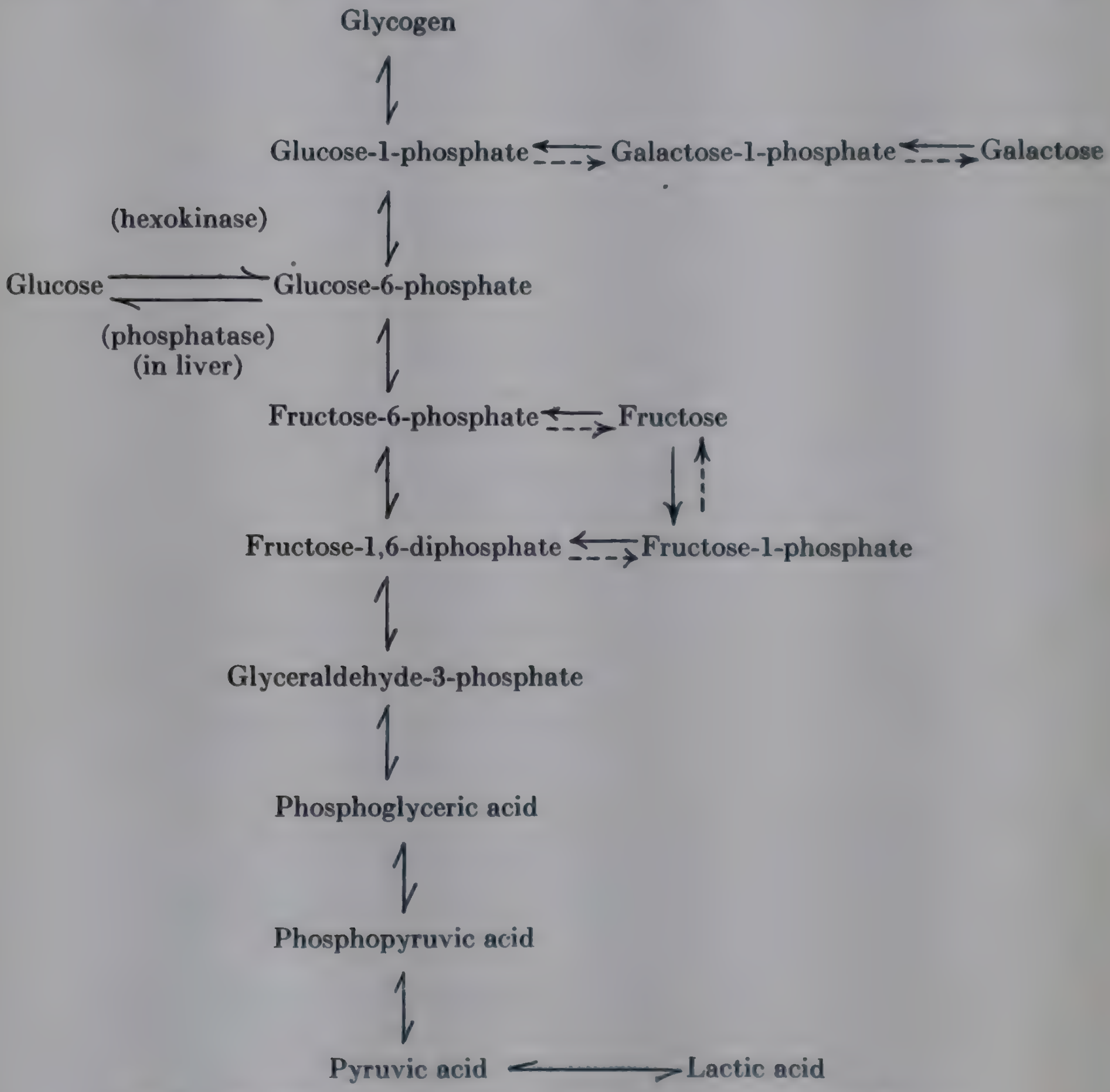
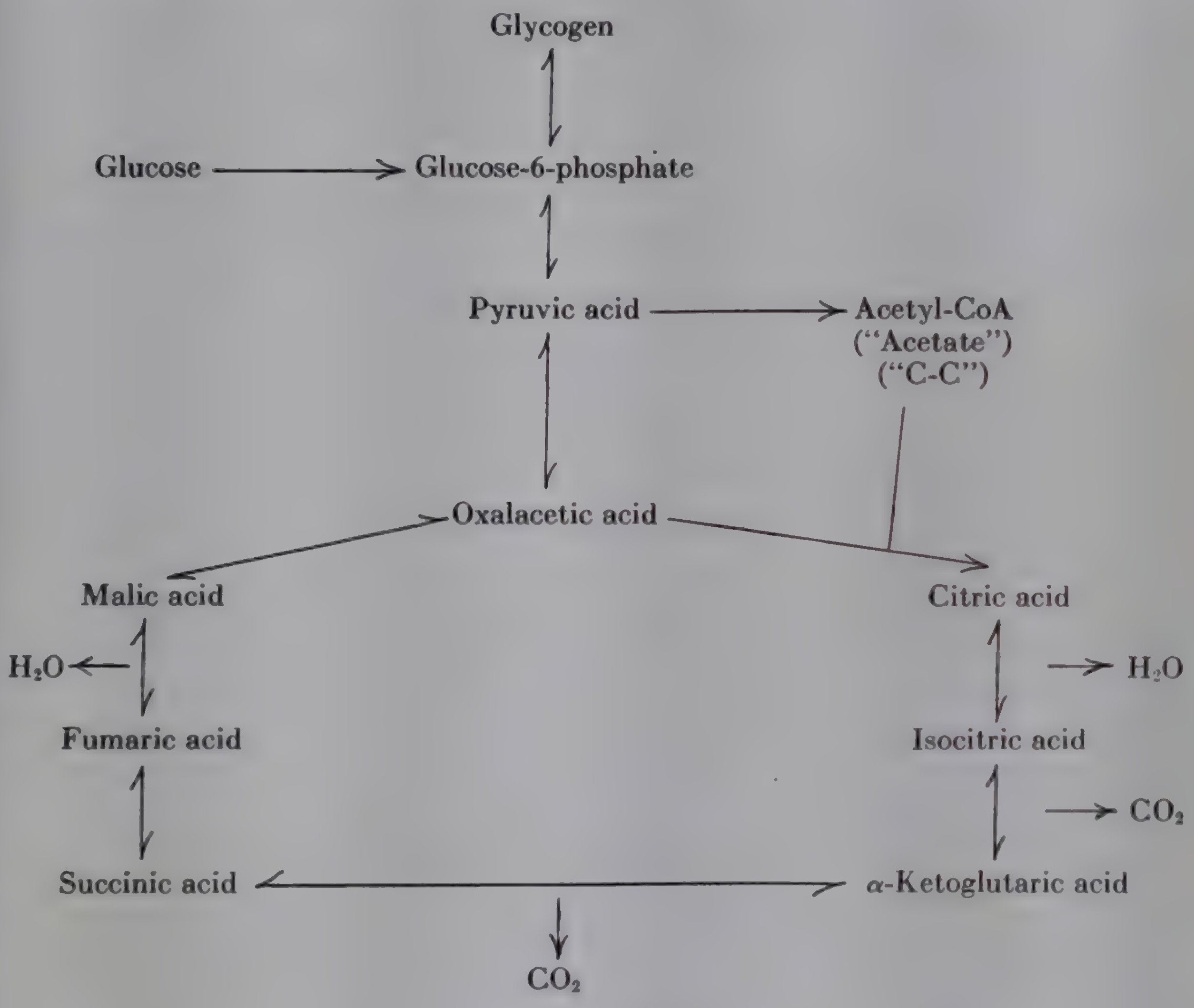

Glycogen
Glucose-1-phosphate
Galactose-1-phosphate
Galactose
(hexokinase)
Glucose
Glucose-6-phosphate
(phosphatase)
(in liver)
Fructose-6-phosphate
Fructose
Fructose-1,6-diphosphate
Fructose-1-phosphate
Glyceraldehyde-3-phosphate
Phosphoglyceric acid
Phosphopyruvic acid
Pyruvic acid
Lactic acid

Fig. 2. Anaerobic metabolism (glycolysis) of glucose and related hexoses.

Under normal conditions of oxygen supply in most tissues, the intermediary metabolism of glucose follows the path through pyruvic acid and the tricarboxylic acid (Krebs; citric acid) cycle (Fig. 3), with the ultimate production of CO_2 and H_2O. However, lactic acid is produced physiologically during the anaerobic phase of muscle contraction and in excess during strenous physical exercise, passing into the blood stream, from which it is removed and converted to glucose or glycogen by the liver. Lactic acid is utilized for energy production (oxidation to CO_2 and H_2O) by heart muscle, but not to a significant extent by other muscles, under physiological conditions.


Glycogen
Glucose
Glucose-6-phosphate
Pyruvic acid
Acetyl-CoA
("Acetate")
("C-C")
Oxalacetic acid
Malic acid
Citric acid
H₂O
H₂O
Fumaric acid
Isocitric acid
CO₂
Succinic acid
α-Ketoglutaric acid
CO₂

Over-all reaction: Pyruvic acid($CH_3COCOOH$) + 5(O) $\longrightarrow$ $2H_2O + 3CO_2$

Fig. 3. Aerobic metabolism of carbohydrate. Tricarboxylic acid (citric acid) (Krebs) cycle.

Aerobic Metabolism of Glucose (Fig. 3). The major route of aerobic metabolism of glucose is identical with the anaerobic through the stage of pyruvic acid. In the presence of adequate amounts of oxygen, the latter is oxidized (and decarboxylated) to CO_2 and H_2O, instead of being reduced to lactic acid. The several steps involved are collectively designated the tricarboxylic or citric acid cycle (Krebs cycle), and are responsible for the production of over 90 per cent of the energy derived from the metabolism of carbohydrate. The over-all oxidation of pyruvic acid may be represented as follows:

$$CH_3\ CO\ COOH + 5O \rightarrow 2H_2O + 3CO_2$$

Certain features of this process should be pointed out because they have an important bearing on the metabolic interrelations of carbohydrates, fatty acids (fats), and amino acids (proteins).

The major portion of pyruvic acid undergoes oxidative decarboxylation to a 2-carbon (acetyl) fragment which may be regarded as acetate, although it is in reality "active acetate," or acetyl-CoA, a compound of acetic acid and coenzyme A, which contains pantothenic acid (one of the B vitamins). This initial step, i.e., oxidative decarboxylation of pyruvic acid, requires other B vitamins, i.e., thiamine pyrophosphate (p. 457) and lipoic acid, as coenzymes. Consequently, thiamine deficiency (as in beri-beri) results in accumulation of excessive amounts of pyruvic (and lactic) acid in the body fluids (p. 56).

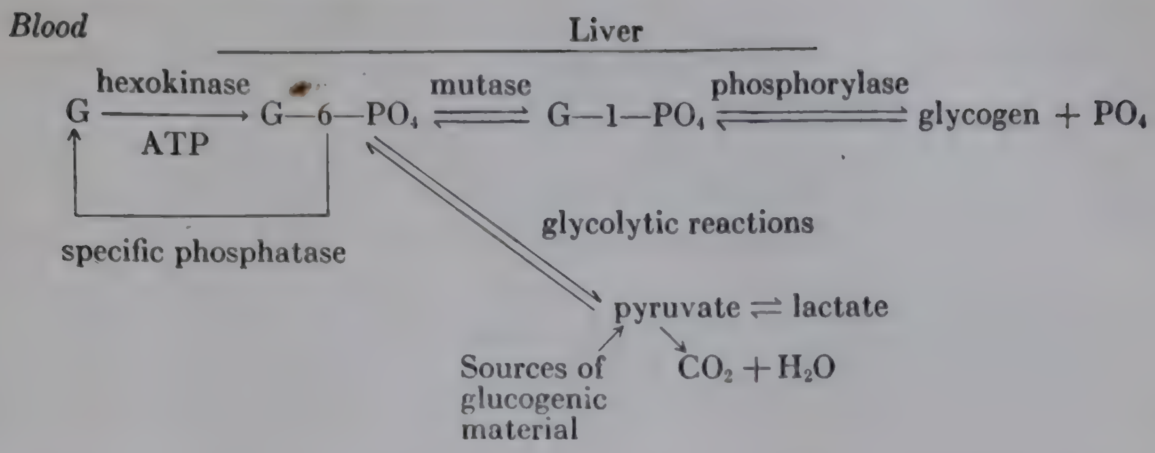
This 2-carbon, acetyl fragment is formed also from fatty acids and certain amino acids, and represents one of the major junctions in the integration of the metabolism of carbohydrate, fat and protein (Fig. 5). This fragment condenses with oxalacetate (from pyruvate and amino acids), forming citrate. After a series of reactions involving oxidation and decarboxylation, a molecule of oxalacetate remains, and, in effect, the 2-carbon fragment with which it had originally condensed has been transformed to CO_2 and H_2O in its passage through the cycle.

Although fatty acids are eventually metabolized via the same acetyl fragments as carbohydrate, they cannot increase the quantity of the latter material in the body since the reaction, pyruvate to acetate, is irreversible in man. Certain amino acids, such as alanine, aspartic acid, and glutamic acid, are directly or indirectly convertible (oxidative deamination, transamination) to Krebs cycle intermediates (Fig. 3). Inasmuch as the reactions within the cycle and those of glycolysis are reversible (in the liver), such amino acids can produce new molecules of glucose or glycogen, and are therefore "glucogenic." These reactions form the basis for the phenomenon of gluconeogenesis (p. 9).

The fact that a molecule of oxalacetate is required for the oxidation of acetyl fragments, including those from fatty acids, makes it obvious that normal carbohydrate metabolism, which is the main source of supply of oxalacetate, is a prerequisite to the complete oxidation of fatty acids. In the absence of an adequate supply of oxalacetate (as in carbohydrate deprivation), a larger proportion of acetyl fragments is diverted (in liver) to the formation of acetoacetic acid ("ketone bodies") (p. 94) and cholesterol (p. 114). It is probable, too, that carbohydrate unavailability, in addition to causing acceleration of catabolism of fatty acids and amino acids for energy production, leads to a further breakdown of amino acids to form members of the tricarboxylic acid cycle, upon the proper functioning of which depends a major part of the metabolism of the body.

ROLE OF LIVER IN CARBOHYDRATE METABOLISM

Glycogenesis and Glycogenolysis. In addition to the general reactions of carbohydrate metabolism shown in Figure 3, the liver is able to perform certain transformations peculiar to itself. One of these is the glucose-6-phosphatase reaction. As a result of the presence of this specific phosphatase in liver, the following equilibria obtain:



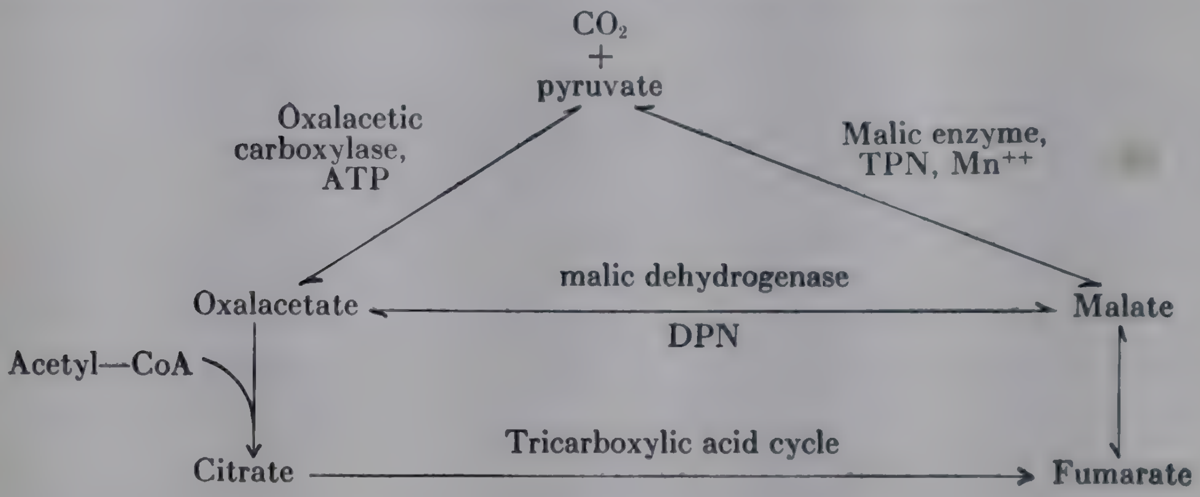
Glycogenesis in liver can occur from blood glucose or from any substance capable of giving rise to pyruvate. In the latter category are glycerol, blood lactate (from muscle, p. 12), and the glucogenic amino acids (p. 136).

Owing to the glucose-6-phosphatase, liver glycogen can contribute directly to blood sugar (glycogenolysis, p. 20) by way of glucose-1-phosphate and glucose-6-phosphate. (In certain tissues, nonspecific phosphatases may substitute for glucose-6-phosphatase.) It should be pointed out that, according to the above scheme, glucogenic substances in liver can form blood sugar without necessarily passing through the stage of liver glycogen.

The concentration of glycogen in the liver at any time is the resultant of glycogenolysis and the various routes of glycogenesis, the influx of dietary carbohydrate being one of the most important of the latter. Human liver contains, on the average, some 5 to 6 per cent glycogen. In dogs, the concentration may rise to 10 to 15 per cent after heavy carbohydrate feeding, and falls to less than 1 per cent on fasting. In certain cases of glycogen storage disease in human beings (von Gierke's disease), a deficiency of glucose-6-phosphatase has been found in the liver. Inability to carry out glycogenolysis, in the face of undiminished glycogenesis from blood sugar, probably accounts for the abnormal accumulation of liver glycogen in these subjects. The level of the blood sugar itself is a major factor in determining the relative rates of glycogenesis and glycogenolysis (p. 22). Endocrine influence on the several reactions involved is discussed elsewhere (p. 14).

Gluconeogenesis. The major conversions of other types of foodstuffs into carbohydrate occur in the liver. The pathway whereby glycerol may be converted to glucose was indicated previously. This, however, is a relatively minor source of carbohydrate; the chief gluconeogenetic substances are the amino acids. The nitrogen-free carbon skeletons of certain amino acids, as will be shown elsewhere (p. 12), are capable of conversion to pyruvic acid, from which point glucose or glycogen can be formed by reversal of the glycolytic reactions. This process is accelerated under conditions of carbohydrate deprivation. Gluconeogenesis from amino acids is under the control of adrenal cortical hormones (p. 16).

Assimilation of CO_2. Although carbon dioxide is usually considered a waste product of metabolism, ample evidence has been accumulated in recent years to support the view that this substance is an important building-block in anabolic reactions. The reactions which appear to account for much of the CO_2 assimilatory activity of the animal body occur largely in liver, and may be regarded as adjuncts to the reactions of the tricarboxylic acid cycle. One such reaction in liver is the reversible β-decarboxylation of oxalacetic to pyruvic acid, which, however, is known to be much more complex than appears on the surface. A second reaction is due to the "malic enzyme," which catalyzes a simultaneous oxidation and decarboxylation of malic to pyruvic acid, completely by-passing the stage of oxalacetic acid. A tentative scheme of these reactions and their relationship to the tricarboxylic acid cycle proper is shown below:



The physiological significance of the above reactions would seem to be that the liver, in contrast to other tissues in which such reactions are not so well developed, can readily synthesize its own supply of catalytic acids for the cycle (e.g., oxalacetic), as long as a source of pyruvate is available. (CO_2, of course, is always in plentiful supply in any living tissue.) During the metabolism of carbohydrate, pyruvic acid is formed as a normal intermediate, hence the aerobic catabolism of carbohydrate is never retarded as a consequence of lack of oxalacetate. In the case of fatty acid catabolism, on the other hand, the provision of adequate oxalacetate from carbohydrate metabolism may be an important factor in determining whether the fatty acids will be completely oxidized to CO_2 or shunted into ketone bodies as a side reaction (p. 94).

MUSCLE IN CARBOHYDRATE METABOLISM

The ensuing discussion is concerned chiefly with skeletal muscle. Certain peculiarities of other muscles should be noted, however. Cardiac muscle differs from skeletal in its ability to oxidize blood lactic acid for energy production. Diaphragm, in contrast to skeletal muscle, is able to reverse the glycolytic sequence of reactions and synthesize glycogen from pyruvate or lactate.

Glycogenesis. The formation of muscle glycogen from blood glucose follows the same path as in liver. The absence of glucose-6-phosphatase from muscle, however, makes it impossible for muscle glycogen to contribute directly to blood sugar; consequently glycogenolysis, as ordinarily defined, does not occur in muscle. It should be noted that nonspecific phosphatases, which might be expected to catalyze the same reaction, are also virtually absent from muscle.

The level of muscle glycogen varies with the dietary intake of carbohydrate and its precursors, but does not fluctuate as widely as liver glycogen. The average concentration in human muscle is 0.4 to 0.6 per cent. In general, muscle glycogen is subject to the same hormonal controls as liver glycogen, except in the case of the hyperglycemic factor of the pancreas (p. 17). Muscle glycogen is probably not formed to any extent by gluconeogenesis; its sole source seems to be blood glucose.

Glycolysis. The glycolytic reactions in muscle follow the general course outlined previously. In muscle, however, the process seems to be irreversible, since experiments have shown that isotopically labeled lactic acid is not converted to labeled glycogen in the perfused muscle, although it is readily oxidized to CO_2. The reason for the irreversibility has not been definitely established, although the reported deficiency in skeletal muscle of fructose-1,6-diphosphatase, a necessary enzyme on the return path, may be relevant. Another consideration is the great permeability of muscle to lactic acid; any that is formed (under anaerobic conditions) is readily lost to the blood. This lactic acid is taken up by the liver, and may be converted to glycogen or blood glucose, the complete route of: blood glucose ⟶ muscle glycogen ⟶ lactate ⟶ liver glucose-6-phosphate ⟶ blood glucose, being known as the Cori cycle.

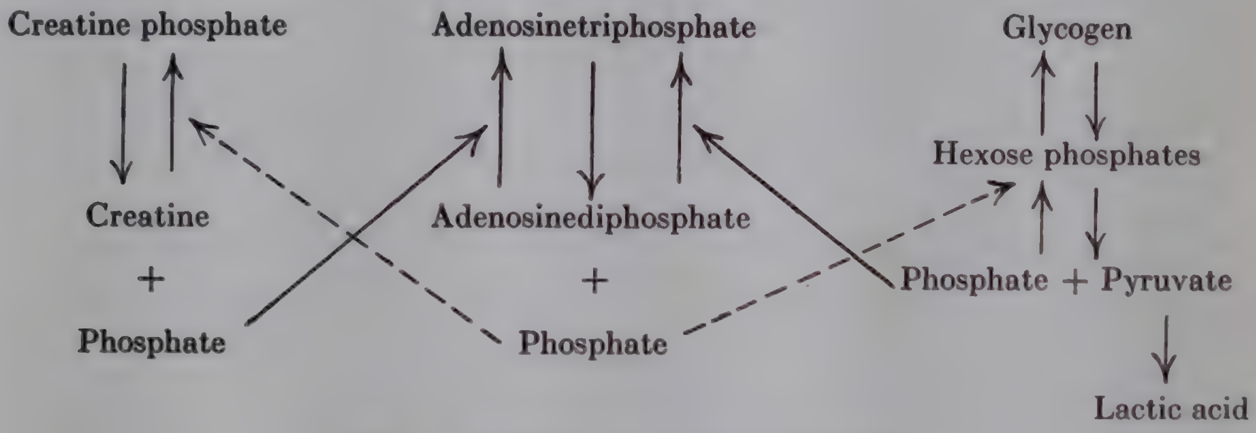


Fig. 4. Chemistry of muscle contraction.

Muscle Contraction. As a form of mechanical work, it is to be expected that muscle contraction would use as its source of energy that common currency of bioenergetics, adenosinetriphosphate (ATP) (p. 196). It is generally agreed that the contractile phenomenon involves a reaction between ATP and actomyosin, a contractile protein, resulting in the formation of ADP and inorganic phosphate, the energy of hydrolysis of the terminal phosphate linkage of ATP being transformed to the mechanical work of contraction (Fig. 4). Although the ATP may be derived from other energy-producing reactions, such as oxidation of fatty acids and ketone bodies, it is probable that oxidation of carbohydrate is the main source of supply in muscle.

Concomitantly with the utilization of ATP in the contractile act, a series of recovery processes occurs. Phosphocreatine, the most important storage form of high-energy phosphate in muscle (p. 150), reacts with ADP. This is accompanied or shortly followed by glycolysis (anaerobic) of stored muscle glycogen or glucose that has entered from the blood, the end product (under relatively anaerobic conditions) being lactic acid. This largely passes into the blood for utilization elsewhere, e.g., reconversion to glycogen or glucose in the liver (p. 9). However, the comparatively low rate of regeneration of ATP during glycolysis (p. 5) would not permit continuous muscular exertion for prolonged periods. This requirement is met by aerobic recovery processes, actually practically synonymous with the operation of the tricarboxylic acid cycle, which provides for rapid regeneration of ATP. When the muscle is at rest, the usual oxidative catabolism of carbohydrate (and probably other substances) produces a surplus of ATP, the excess of high-energy phosphate being stored as phosphocreatine

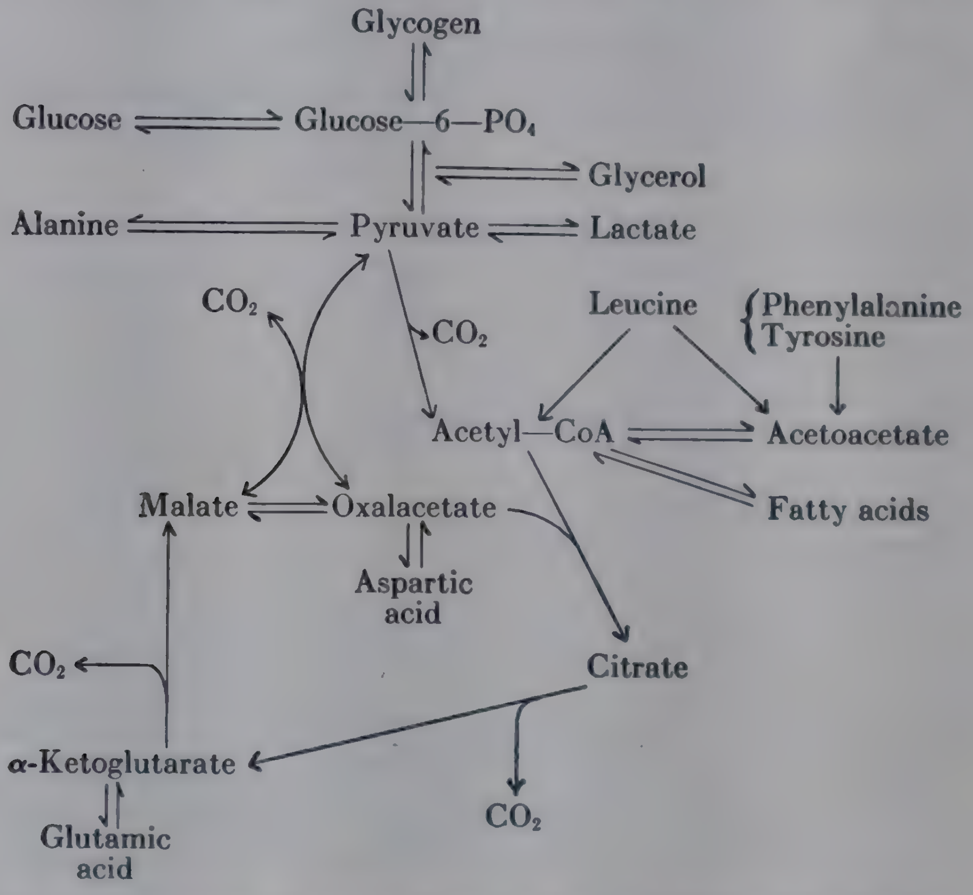

Glycogen
Glucose
Glucose—6—PO₄
Glycerol
Alanine
Pyruvate
Lactate
CO₂
CO₂
Leucine
Phenylalanine
Tyrosine
Acetyl—CoA
Acetoacetate
Fatty acids
Malate
Oxalacetate
Aspartic acid
CO₂
Citrate
α-Ketoglutarate
CO₂
Glutamic acid

Fig. 5. Interrelation of carbohydrate, protein, and fatty acid metabolism.

(creatine + ATP → phosphocreatine + ADP), thus providing a ready reservoir of potential ATP for future contractions.

INTERRELATION OF CARBOHYDRATE, LIPID, AND PROTEIN METABOLISM

One of the great contributions of isotopic studies has been the demonstration of the intimate interrelation of carbohydrate, lipid and protein metabolism. This has led to a better understanding of many abnormalities that occur in disease states.

The metabolism of the three major foodstuffs exhibits many points of interaction (Fig. 5). Metabolites of carbohydrate can form fatty acids and the carbon skeletons of certain amino acids, and many amino acids can be converted to carbohydrate. Fragments from all classes of foodstuffs are eventually channeled into a common pathway of aerobic catabolism, the tricarboxylic acid cycle, for ultimate conversion to CO_2 and H_2O, and liberation of energy.

The most important common junction in the metabolism of fatty acids and carbohydrate is the 2-carbon fragment ("active" acetate; acetyl-CoA), which is formed (*a*) by oxidative decarboxylation of pyruvate (which is formed from carbohydrates and certain amino acids (glucogenic)), (*b*) by oxidation of fatty acids, and (*c*) directly from certain amino acids (ketogenic amino acids). Moreover, the majority of amino acids are directly or indirectly convertible to carbohydrate intermediates (p. 138), and, since, in the liver, the main reaction sequences of carbohydrate metabolism are reversible, these amino acids can form new molecules of glycogen or glucose (glucogenic amino acids). Likewise, by reversal of these processes, carbohydrate is convertible to (*a*) fatty acids (via "acetate"), and, therefore, into neutral fat (fatty acids plus glycerol), which itself can be formed from carbohydrate (p. 88), and (*b*) nonessential amino acids, by amination of α-keto acid intermediates (e.g., pyruvate → alanine; oxalacetate → aspartic acid; α-ketoglutaric acid → glutamic acid).

In the liver, each "acetate" fragment can take one of four main metabolic pathways: (*a*) condensation with oxalacetate, forming citrate, and thus entering the tricarboxylic acid cycle for conversion to CO_2 and H_2O; (*b*) synthesis of fatty acids; (*c*) synthesis of cholesterol; (*d*) condensation with another "acetate" molecule, to form acetoacetate (ketogenesis). The last indicated pathway (ketogenesis) is not available in extrahepatic tissues.

Inasmuch as a molecule of oxalacetate is required to carry each molecule of "acetate" through the tricarboxylic acid cycle, i.e., to permit its oxidation to CO_2 and H_2O, and since carbohydrate is the main source of oxalacetate, it is obvious that normal carbohydrate metabolism is a prerequisite to the complete oxidation of fatty acids. This is particularly the case in the liver, where, although oxalacetate is formed from certain amino acids, it is rapidly decarboxylated to pyruvic acid unless the latter is continually provided in adequate amounts from carbohydrate. In the absence of an adequate supply of oxalacetate (e.g., carbohydrate deprivation, diabetes mellitus), the "acetate" molecules, deprived of this major pathway of utilization, are diverted in excess (in the liver) into ketone body formation and cholesterol synthesis (p. 94). Carbohydrate, taken in excess of the requirement for energy, is readily converted to and stored as fat (via "acetate" and fatty acid synthesis).

Certain hormonal mechanisms are integrated to a common purpose in this connection, i.e., to maintain an adequate supply of energy. The system fundamentally involved is the balance between insulin, on the one hand, and the adrenal 11-oxysteroids and pituitary growth hormone on the other. The pertinent actions of these hormones are outlined in detail elsewhere (pp. 14, 16, 17). It is necessary here merely to indicate the over-all metabolic consequences of imbalance of these factors. Insulin deficiency, absolute (i.e., pancreatic islet insufficiency) or relative (i.e., adrenocortical or anterior pituitary excess), results in: (*a*) depression of carbohydrate utilization (i.e., inadequate provision of oxalacetate from carbohydrate); (*b*) stimulation of mobilization of fat from depot stores (pituitary and adrenal action), with hyperlipemia; (*c*) excessive formation of "acetate" from fatty acids; (*d*) excessive diversion of "acetate" into ketogenesis (in liver); (*e*) excessive cholesterol synthesis (in liver); and (*f*) excessive protein catabolism (adrenal action), with increased gluconeogenesis and negative nitrogen balance. Similar phenomena follow undue restriction of carbohydrate intake.

ENDOCRINE INFLUENCES IN CARBOHYDRATE METABOLISM

The several processes concerned with the supply of glucose to the blood by the liver and with its utilization in the tissues must be regulated and integrated, not only with one another, but also with certain phases of the metabolism of proteins and lipids. Endocrine organs play an important role in this homeostatic mechanism. These may be placed in two categories in this connection: (1) those which exert a fundamental regulatory influence, their normal function being essential for normal carbohydrate metabolism (viz., pancreatic islet cells, adrenal cortex, anterior pituitary); (2) those which influence carbohydrate metabolism, but are not essential for its regulation (viz., adrenal medulla, thyroid).

Insulin. The over-all effects of insulin on the organism are illustrated by certain observable metabolic consequences of (1) pancreatectomy and (2) administration of insulin to normal animals.

1. *Pancreatectomy* is followed by:
   a. Decreased glucose tolerance.
   b. Hyperglycemia and glycosuria.
   c. Depletion of glycogen in liver and muscle.
   d. Decreased rate of oxidation of glucose (decreased R.Q.).
   e. Increased gluconeogenesis; increased urinary nitrogen.
   f. Increased mobilization of depot fat; increased ketogenesis.
   g. Increase in cholesterol and other lipids in the plasma.
2. *Insulin administration* is followed by:
   a. Decrease in blood sugar.
   b. Increased oxidation of glucose (increased R.Q.).
   c. Increased muscle glycogen.

The most important known actions of insulin are as follows:

1. Acceleration of glucose oxidation.
2. Acceleration of conversion of glucose to fat.
3. Increase in liver glycogen formation and/or inhibition of hepatic glycogenolysis by other hormones.
4. Inhibition of gluconeogenesis in liver.
5. Inhibition of excessive ketogenesis.

Increased utilization of glucose by oxidation in the tissues, for lipogenesis and for glycogen formation in muscle, accelerates its removal from the blood stream. The ability of the liver to retain more glycogen than at comparable levels of blood sugar in the absence of increased amounts of insulin decreases the rate of entrance of glucose into the blood stream. Both of these phenomena contribute to the resulting fall in blood sugar concentration. It is not known whether insulin exerts a direct influence on the formation of liver glycogen or whether it opposes hepatic glycogenolysis by other hormones. This tendency for the liver to retain glycogen is masked by the existing hypoglycemia, which itself accelerates hepatic glycogenolysis.

There is evidence that insulin antagonizes the inhibitory influence of an anterior pituitary factor and adrenocortical hormones on the hexokinase reaction, thus serving to accelerate it. Inasmuch as this is the first step in the utilization of glucose, for both oxidation and glycogen synthesis, this phenomenon may represent one of the fundamental effects of insulin in this connection. Oxidation of fructose by livers of diabetic animals, contrary to that of glucose, is normal, thus localizing the metabolic block (and the action of insulin) in the conversion of glucose to fructose-6-phosphate, which includes the hexokinase reaction. It has been suggested that one of the fundamental actions of insulin may be to promote the passage of glucose from the extracellular fluid into the cell. This is, of course, a prerequisite for its metabolic utilization. There is evidence that insulin and other hormones combine chemically and in some instances competitively (e.g., insulin and an anterior pituitary hormone) with muscle. It has been suggested that such competitive interaction between hormones with antagonistic metabolic effects for sites of chemical combination with the tissues of specific target organs may constitute the basis for the action of hormonal regulatory mechanisms.

One of the major functions of insulin is to promote conversion of carbohydrate to fat (i.e., long-chain fatty acids). In the livers of diabetic animals, the conversion of glucose to fatty acids is depressed to an even greater extent than is its oxidation. Moreover, production of fatty acids from both acetate and fructose is also depressed, although their oxidation proceeds normally. These facts indicate that insulin facilitates the conversion of a 2-carbon-like metabolic intermediate to fatty acid. On the other hand, insulin is not required for the conversion of acetate to cholesterol.

In diabetes (insulin deficiency), there is an increase in liver fat, excessive ketogenesis, hyperlipemia, and hypercholesterolemia. The increase in fat in the blood and liver is due to excessive mobilization of depot fat, in consequence of the state of hormonal imbalance in the direction of relative excess of adrenocortical and anterior pituitary hormones. In the absence of an adequate supply of insulin, the catabolism of fatty acids, which is unimpaired, results in the production of 2-carbon fragments which cannot be utilized adequately for synthesis of long-chain fatty acids, which are present in amounts exceeding the body's capacity for oxidation. They are diverted, therefore, in increased amounts to acetoacetate (excessive ketogenesis) and cholesterol (hypercholesterolemia).

The hormonal imbalance referred to above (adrenocortical preponderance) results also in stimulation of increased glucose production from amino acids (excessive gluconeogenesis) with associated increased protein catabolism. This increased production of glucose, together with its decreased utilization, contributes to the hyperglycemia associated with insulin deficiency.

Stimulation of glucose utilization, by administration of either glucose or insulin, is accompanied by a decrease in the concentration of potassium and inorganic phosphate in the blood. These electrolyte changes are apparently associated with two phenomena: (1) acceleration of entrance of glucose (or other hexoses) into the tissue cells, and (2) deposition of K and PO_4 with glycogen in liver and muscles. The closely parallel changes in blood glucose, K, and PO_4 that follow administration of insulin are apparently dependent upon common cellular metabolic mechanisms, probably involving phosphorylation processes.

Adrenocortical Hormones. The fasted, untreated, adrenalectomized animal (or patient with Addison's disease) exhibits the following changes in carbohydrate metabolism: (1) striking decrease in liver glycogen; (2) less marked decrease in muscle glycogen; (3) hypoglycemia; (4) decreased intestinal absorption of glucose (corrected by administration of NaCl). These changes do not occur if a sufficient quantity of carbohydrate is given, and are prevented or corrected by administration of 11-oxygenated adrenal hormones. 17-Hydroxycorticosterone (Compound F) is three to five times as potent in this respect as corticosterone or 11-dehydrocorticosterone (Compound A), and 11-dehydro-17-hydroxycorticosterone (Compound E; cortisone) two to three times as potent. There are other indications of the influence of adrenocortical hormones on carbohydrate metabolism:

1. Adrenalectomized animals exhibit increased sensitivity to insulin.
2. Adrenalectomy results in amelioration of diabetes produced by pancreatectomy or alloxan. Administration of 11-oxygenated adrenal hormones or cortical extracts aggravates the diabetes.
3. Administration of cortical extract or 11-oxygenated adrenal hormones causes a rise in blood sugar, liver glycogen, and total body carbohydrate, glycogen formation being accelerated and its breakdown to glucose retarded.

The adrenocortical deficiency manifestations are related to two fundamental phenomena: (1) increased oxidation of glucose and (2) decreased gluconeogenesis from body protein (p. 9). Conversely, cortical hormones active in this sphere diminish oxidation of glucose and increase gluconeogenesis from body protein. One point of action of these hormones is apparently the first reaction in the course of utilization of glucose, i.e., its phosphorylation to glucose-6-phosphate, catalyzed by the enzyme, hexokinase (hexokinase reaction). There is evidence that adrenocortical hormones enhance the inhibitory influence of the anterior pituitary on this reaction, an effect which is counteracted by insulin. This inhibitory effect on glucose oxidation is reflected in a decrease in the respiratory quotient (R.Q.), which, accordingly, is increased in adrenalectomized animals.

Anterior Pituitary Factors. The adrenocorticotrophic (ACTH) and thyrotrophic (TSH) hormones, by stimulating secretion by their respective target organs (adrenal cortex; thyroid), produce effects on carbohydrate metabolism identical with those produced by adrenocortical hormones and thyroxine. In addition, unfractionated anterior pituitary extracts cause a rise in blood sugar and a decrease in R.Q. in adrenalectomized and thyroidectomized animals, which, therefore, cannot be due to ACTH or TSH. There is also an increase in muscle glycogen ("glycostatic" action).

The "diabetogenic" action of such extracts (hyperglycemia, decreased R.Q.)

has been attributed to the growth hormone, but may be due, in part at least, to an unidentified factor separable from this principle. These effects are apparently dependent upon inhibition of utilization of glucose. Although the underlying mechanisms have not been established definitely, there is evidence that the following may be involved: (*a*) inhibition of the hexokinase reaction (enhanced by adrenocortical hormones and counteracted by insulin); (*b*) interference in the glycolytic pathway at some point between glucose-6-phosphate and pyruvic acid, i.e., beyond the hexokinase reaction; (*c*) inhibition of uptake of glucose by tissue cells (counteracted by insulin).

Epinephrine. Epinephrine produces an increase in blood sugar and in blood lactic acid. These are due to acceleration of glycogenolysis in the liver and muscles, respectively, and are accompanied by a decrease in the glycogen content of these structures. The mechanism involved is apparently stimulation of phosphorylase activity (reactivation of inactivated phosphorylase). There is some evidence, too, that epinephrine may diminish the uptake of glucose by tissue cells, thus interfering with its utilization.

Thyroid Hormone. Thyroxine accelerates hepatic glycogenolysis, with consequent rise in blood sugar. This effect is partially offset by simultaneously increased utilization of glucose incident to the acceleration of metabolism and blood flow. Thyroxine also increases the rate of absorption of hexoses from the intestine.

Hyperglycemic Factor of Pancreas (HGF; Glucagon). A factor is present in extracts of the pancreas and of the gastric mucosa of the dog, as well as in most commercial insulin preparations, which causes an increase in blood sugar when injected intravenously. It has been designated the "hyperglycemic factor" (HG) of the pancreas, or glucagon. It exhibits protein characteristics, is apparently formed in the alpha cells of the islets, and is regarded as a hormone.

When purified HG preparations, free of insulin, are injected intravenously, the blood sugar begins to rise immediately, reaches a maximum in about thirty minutes, and falls to the original level in about an hour. Liver slices incubated with this factor show a decrease in glycogen and an increase in glucose-1- and glucose-6-phosphates. This action of the HG factor is due to increased phosphorylase activity. Epinephrine and, to a lesser extent, other sympathomimetic amines, exert the same action; however, the pancreatic HG factor does not cause the increase in blood lactic acid observed after administration of epinephrine.

It is believed that liver phosphorylase undergoes continual enzymatic inactivation and resynthesis (activation) and that the pancreatic hyperglycemic factor (and also epinephrine) accelerates its resynthesis (reactivation). The exact role, if any, of this agent in the normal regulation of carbohydrate metabolism and of blood sugar concentration is not known.

NORMAL POSTABSORPTIVE BLOOD SUGAR

In the resting, postabsorptive state (overnight fast), glucose is present in systemic venous blood in the following concentrations: whole blood, 60 to 100 mg./100 cc.; plasma, 70 to 110 mg./100 cc. There is no clinically significant sex or age difference, although average values are somewhat higher in elderly subjects. Certain analytical procedures in common use yield higher values, i.e., 80 to 120 mg./100 cc. whole blood, because they include also non-sugar reducing substances, e.g., thioneine and glutathione, present chiefly in the erythrocytes. In the fasting state, the concentration of glucose in arterial (or capillary) blood is slightly higher (2 to 10 mg./100 cc.) than in venous blood, owing to its continual passage into and utilization by tissue cells. This "arterial-venous blood sugar difference" is increased under conditions of decreased blood flow or increased glucose utilization, e.g., following administration of carbohydrate (with rise in blood sugar) or of insulin. In man, glucose is distributed equally in the water of the plasma and erythrocytes. The lower concentration in the cells as compared to plasma, per unit volume, is due to their lower water content (because of large amount of hemoglobin). This uniform distribution of glucose between erythrocytes and plasma does not occur in other mammalian species (except anthropoids).

Owing to the operation of an efficient regulatory mechanism, the postabsorptive blood sugar concentration is maintained within the limits indicated in normal subjects except under unusual circumstances. What deviations do occur are usually relatively slight and transitory. Pain and emotional excitement (especially apprehension, anxiety, fear, anger) produce a brief rise (10–20 mg./100 cc.), due to acceleration of hepatic glycogenolysis resulting from increased epinephrine secretion. As the blood sugar rises, insulin secretion is stimulated, which, together with the accelerated blood flow induced by the epinephrine, results in increased utilization (i.e., removal) of glucose by the tissue cells and, consequently, prompt fall in blood sugar. The same may occur with brief, strenuous exercise (increased hepatic glycogenolysis), but may here, too, be dependent largely on the accompanying emotional excitement. Protracted strenuous exercise (e.g., marathon race) may at times result in a fall in blood sugar as a result of depletion of hepatic glycogen. During a prolonged fast in subjects whose activity is maintained, the blood sugar falls somewhat after about 2 days, usually reaching a minimum at 4 to 6 days (average drop 15–30 mg./100 cc.), occasionally falling as low as 40 to 50 mg./100 cc. The fall is of greater degree and occurs more promptly in infants than in adults (greater utilization of glucose and faster depletion of hepatic glycogen). With continued fasting, the blood sugar rises gradually during the second week to approximately the original level. The initial drop is due to depletion of preformed hepatic glycogen, which is necessarily maintained subsequently solely by gluconeogenesis from tissue protein (small amount from lactic acid). The gradual return to normal is effected by the homeostatic mechanism; important adjustments include decreased secretion of insulin (due to hypoglycemia) and thyroxine (due to starvation), with consequent decrease in utilization of glucose, and increased adrenocortical activity (due to stress of starvation), with consequent increased gluconeogenesis.

Glucose in Body Fluids Other than Blood. Glucose diffuses readily across blood and lymph capillary walls and, therefore, in the postabsorptive state, is equally distributed in the water of the blood plasma and interstitial fluid (also synovial fluid). Its concentration in cerebrospinal fluid removed from the ventricles is about 10 to 20 mg./100 cc. lower, and that in the lumbar fluid about 20 to 30 mg./100 cc. lower than in the blood plasma obtained simultaneously (postabsorptive state). The glucose content of the aqueous humor of the eye is also somewhat lower than that of blood plasma, per unit of water. These facts support the view that the cerebrospinal fluid and aqueous humor are not simple dialysates of blood plasma. Increase in the blood sugar concentration, e.g., after ingestion of glucose, is reflected in the extracellular fluids, the increase in the latter lagging somewhat behind that in the former.

The digestive secretions, with the exception of bile, contain virtually no glucose, even at high blood sugar concentrations. The same is true of milk and of secretions of the prostate and seminal vesicles. Unless the blood sugar is raised to unphysiological levels, viz., by intravenous injection, glucose does not appear in the urine of normal subjects except in negligible quantities. There are occasional exceptions to this statement, e.g., in pregnancy. Although glucose diffuses perfectly through the glomerular capillaries, it undergoes practically complete reabsorption in the renal tubules under normal circumstances, phosphorylation being probably involved in this process, as in its absorption from the intestine.

The concentration of glucose in intracellular water varies considerably in different tissues, but is uniformly considerably lower than in the plasma, except, occasionally, in the liver. The hepatic cells differ from other cells in this connection in that they form glucose (from glycogen) in addition to receiving it from the extracellular fluid. This sugar cannot be stored, as such, to any significant extent in the cells and, consequently, is utilized at least as rapidly as it enters. Indeed, there is evidence that free glucose does not pass readily across the tissue cell membranes by passive diffusion, but is phosphorylated at the surface. Variations in the concentration of glucose in the blood, e.g., after administration of glucose, epinephrine, or insulin, are reflected in that in the tissue cells, more closely in liver and kidney, less so in muscle and brain, in which the concentration of free glucose is relatively low.

Sugars Other than Glucose in Body Fluids. Sugars other than glucose are not present in the blood or interstitial fluid in detectable amounts under normal conditions in the postabsorptive state. In a considerable proportion of normal lactating women, a small portion of the lactose which is formed from glucose in the breast and is excreted in the milk passes into the blood stream, and, not being utilizable by the tissues, is excreted in the urine. However, its concentration in the blood is rarely sufficiently high to permit its detection by ordinary methods. Fructose, galactose, and pentoses are readily absorbed in the intestine from dietary sources. However, under ordinary conditions of intake their removal from the circulation and their utilization proceed so rapidly that they are not detectable in the blood of normal subjects. The same is true in subjects with essential fructosuria and essential pentosuria, in which conditions the excretion of these sugars in the urine presupposes their presence in the blood. Fructose phosphates are continually formed in all cells during the process of glycolysis, but apparently do not leave the cells. Pentoses and galactose also are formed in the organism, from glucose, the former being incorporated in nucleotides and nucleic acids, the latter in galactolipids (and lactose, in lactating breast). However, they, too are either utilized completely *in situ* or enter the blood stream in amounts too small to permit their detection.

After ingestion of supertolerance quantities of fructose or galactose (e.g., 40 gm.) by normal subjects, a sufficient quantity may escape prompt conversion to glycogen in the liver to cause their transitory appearance in the systemic circulation (30–75 minutes) in demonstrable amounts. They are also excreted in the urine under such circumstances, inasmuch as, in contrast to glucose, they do not undergo reabsorption from the glomerular filtrate in the uriniferous tubules. This may occur, too, in subjects with liver damage (i.e., impaired glycogenesis) after ingestion of small amounts of fructose or galactose. An alimentary form of pentosuria is also encountered occasionally, usually without appreciable amounts in the blood.

Lactose is present in milk, being synthesized in abundance by lactating mammary tissues from glucose and galactose, both of which originate in the blood glucose, the galactose, too, being formed *in situ*. Insulin is apparently not involved in this process. Fructose is present in high concentration (300–700 mg./100 cc.) in seminal fluid. It is apparently formed by the seminal vesicular epithelium, this function, in common with others of that structure, being stimulated by androgens (e.g., testosterone) and decreased by castration or hypophysectomy.

REGULATION OF BLOOD GLUCOSE CONCENTRATION

The concentration of glucose in the blood is the resultant of two general factors: (1) the rate of its entrance into and (2) the rate of its removal from the blood stream. In the normal postabsorptive state (no glucose in the intestine), it is probably contributed to the blood solely by the liver (p. 9), entering the hepatic vein at the rate of approximately 115 mg. (± 10%)/sqM./min. Inasmuch as its concentration in the blood remains fairly constant under these conditions (resting, fasting), it must leave the blood, in the capillaries, at approximately the same rate. These two general processes are influenced by a number of factors under physiological conditions.

Rate of Supply of Glucose to Blood. Except for a possible minor contribution by the kidney, which probably does not occur under physiological conditions, the blood glucose may be derived directly from the following sources (Fig. 1, p. 4): (1) hepatic glycogen; (2) amino acids (gluconeogenesis, in liver); (3) other glucose precursors, e.g., lactic acid, fructose, galactose; (4) glucose absorbed from the intestine, which may be incompletely removed from the portal blood by the liver.

HEPATIC GLYCOGENESIS AND GLYCOGENOLYSIS. In the postabsorptive state in a well-nourished person, the most important of the above factors is the liver glycogen. Under physiological conditions the following factors influence the rate of hepatic glycogenesis and glycogenolysis:

(*a*) *Blood Glucose Concentration.* A rise in blood sugar increases, and a fall in blood sugar decreases the rate of hepatic glycogenesis. This results in part perhaps from a "mass action" effect (glucose → glycogen), and also from the incident changes in secretion of insulin by the pancreatic islet cells. A fall in blood sugar to hypoglycemic levels stimulates secretion of epinephrine, with consequent increased hepatic glycogenolysis.

(*b*) *Insulin.* Increase in the amount of insulin reaching the liver increases the rate of hepatic glycogenesis. It is particularly interesting in this connection that the blood glucose concentration is the most important if not the only factor governing secretion of insulin, which increases as the blood sugar rises and decreases as it falls.

(*c*) *Epinephrine.* Increase in the amount of epinephrine reaching the liver increases the rate of hepatic glycogenolysis. Secretion of epinephrine is stimulated by hypoglycemia, however induced, and by a variety of emotional and other factors, including fear, anxiety, apprehension, anger, pain, unusual exertion, etc.

(*d*) *Thyroxine.* Increase in the amount of thyroxine reaching the liver increases the rate of hepatic glycogenolysis. This may occur during exposure to cold.

(*e*) *Muscular Activity.* Vigorous exercise is accompanied by an increase in the rate of hepatic glycogenolysis (epinephrine?).

ABSORPTION FROM INTESTINE. A variable portion of the glucose absorbed from the intestine is removed from the portal blood by the liver (glycogenesis, lipogenesis, oxidation). The remainder passes directly into the systemic circulation. The relative proportions taking these routes depend largely upon (1) the capacity of the liver, at the moment, for further storage of glycogen, and (2) the rate of absorption of glucose from the bowel. The latter is increased by thyroid hormone and is decreased in the presence of increased intestinal motility, the glucose being hurried through the upper intestine, the region of maximal absorption.

GLUCONEOGENESIS. If provided in adequate amount, dietary carbohydrate is the main source of liver glycogen. In carbohydrate restriction, increasing amounts, and in starvation virtually all of the liver glycogen is derived from amino acids. This process of gluconeogenesis (p. 9) is accelerated by the 11-oxygenated adrenal hormones and is retarded by insulin. If adequate amounts of glycogen are present in the liver, glucose may be formed directly by gluconeogenesis and pass into the blood stream.

Rate of Removal of Glucose from Blood. Glucose leaves the blood in all tissues, being utilized by all cells for the production of energy (oxidation), and by certain cells for more specialized purposes, e.g., glycogenesis, lipogenesis, etc. (p. 2). Inasmuch as free glucose cannot be stored as such in cells in significant amounts, the rate of utilization of glucose in the tissues will determine the rate at which it is removed from the blood. Under physiological conditions, this is influenced by the following factors:

(*a*) *Blood Glucose Concentration.* A rise in blood sugar increases and a fall in blood sugar decreases the rate of glucose utilization. As in the case of hepatic glycogenesis, this may be due in part to a "mass action" effect (glucose → end products), and also to the incident changes in insulin secretion.

(*b*) *Insulin.* Increase in the amount of insulin reaching the tissues increases the rate of utilization of glucose for all purposes (oxidation, glycogenesis, lipogenesis). Again, attention is directed to the fact that secretion of insulin increases as the blood sugar rises and decreases as it falls.

(*c*) *Adrenocortical and Pituitary Hormones.* Oxidation of glucose is depressed by a factor (growth hormone?) or factors secreted by the anterior pituitary, and by the 11-oxygenated adrenocortical hormones. Increase in the latter occurs, under physiological conditions, in response to a great variety of "alarming" stimuli (alarm reaction), which include practically all unpleasant emotions (anger, fear, anxiety, etc.) and pain, as well as other types of stress (exposure to cold, physical and mental tension, etc.).

Fundamental Regulatory Mechanism. The processes of hepatic glycogenesis and tissue utilization of glucose are sensitive to relatively slight deviations from the normal blood sugar concentration. As the latter rises, glycogenesis is accelerated and utilization increased, with consequent fall in blood sugar. The reverse occurs as the blood sugar concentration falls. The normal balance between production and utilization of blood sugar at a mean level of circulating (plasma) glucose of approximately 80 mg./100 cc. (70–110 mg.) is therefore dependent upon the sensitivity of these processes to variations above and below this concentration. This level of sensitivity is determined to a considerable extent by the balance between insulin, on the one hand, and hormones of the adrenal cortex and anterior pituitary on the other.

The over-all effect of insulin is to lower the blood sugar, that of the adrenocortical and pituitary factors to raise it. Inasmuch as these two sets of factors are mutually antagonistic in this respect and, indeed, in several aspects of their metabolic actions, it is the ratio between them rather than their absolute amounts that is of primary importance in this connection. With minor differences, decrease in insulin produces much the same effects on carbohydrate metabolism as does increase in adrenocortical hormones or in the appropriate anterior pituitary principles. Similarly, the effects of increase in insulin resemble closely those of decrease in either of the other factors. The fundamental importance of this balance is demonstrated strikingly by the fact that diabetes induced by removal of the pancreas is ameliorated considerably by subsequent hypophysectomy (Houssay preparation) or adrenalectomy.

The processes of hepatic glycogenesis and glycogenolysis and glucose utilization, and also the blood sugar concentration, are continually exposed to disturbing influences under physiological conditions. These include absorption of glucose from the intestine, physical and mental activity, emotional states, etc. The primary effect of the majority of these is a rise in blood sugar. As indicated previously, this automatically results in a net decrease in the delivery of glucose by the liver and acceleration of its utilization by the tissues. There is a simultaneous increase in secretion of insulin, stimulated by the elevated blood sugar concentration, with consequent increase in the ratio of insulin to adrenocortical and anterior pituitary hormones. This change in hormonal balance results in increased hepatic glycogenesis, decreased gluconeogenesis and increased utilization of glucose. The blood sugar falls accordingly. A drop in blood sugar below the normal resting level is accompanied by decreased secretion of insulin, decrease in the ratio of insulin to the antagonistic adrenocortical and pituitary hormones, increased production of blood sugar, and decreased glucose utilization. Accordingly, the blood sugar rises. If the blood sugar falls to hypoglycemic levels, an additional emergency mechanism comes into operation, i.e., stimulation of epinephrine secretion (by hypoglycemia), resulting in acceleration of hepatic glycogenolysis and rise in blood sugar. The increase in epinephrine may also stimulate production of ACTH and, therefore, adrenocortical hormones, causing increased gluconeogenesis.

In the ultimate analysis, the blood sugar concentration regulates itself. Efficient operation of this autoregulation at physiological levels, however, requires a normal balance between insulin and the carbohydrate-active adrenocortical and anterior pituitary hormones, and also normal responsiveness of the pancreatic islet cells to variation in blood sugar. This constitutes the central regulatory mechanism. If it is intact, the blood sugar tends to remain within rather narrow limits in the face of periodic disturbances which may cause temporary fluctuations. On this basis it can be readily understood why conditions of abnormal function of the islands of Langerhans, the adrenal cortex, or the anterior pituitary are accompanied by serious and fundamental disturbances in carbohydrate metabolism and blood sugar regulation. Changes in blood sugar induced by other factors, e.g., epinephrine, thyroxine, exercise, glucose absorption from bowel, etc., are promptly and effectively counteracted by this control mechanism and are therefore usually limited in extent and of rather brief duration.

NORMAL ALIMENTARY REACTION (ABSORPTIVE RESPONSE)

Sugar Tolerance

Ingestion of glucose, starch, and, to a lesser degree, other carbohydrates (fructose, galactose) or protein (but not fat), is followed, in normal subjects, by a rise in blood sugar. The degree of elevation depends somewhat upon the amount of glucose contained in or produced from the ingested material, but this relationship is by no means quantitative. This "alimentary reaction" forms the basis for the carbohydrate tolerance tests commonly employed for the detection of disturbances of carbohydrate metabolism. The characteristics of a normal response may be illustrated by describing the changes which follow ingestion of 100 gm. of glucose (or 1.75 gm./kg.) (oral glucose tolerance test). Samples of blood are obtained before, and at intervals (Fig. 6) after administration of the glucose.

The characteristic changes in venous blood are as follows: (*a*) A sharp rise to a peak, averaging about 50 per cent above the fasting level, within 30 to 60 minutes. The extent of the rise varies considerably, but the maximum should not exceed 170 mg./100 ml. in normal subjects, regardless of the initial level. This rise is due directly to the glucose absorbed from the intestine, which temporarily exceeds the capacity of the liver and tissues for removing it. As the blood sugar concentration increases, however, the regulatory mechanism (p. 22) comes into play; hepatic glycogenesis is accelerated and glucose utilization increases; insulin secretion is stimulated, accentuating these phenomena. The peak of the curve is reached when utilization is accelerated to the point where the glucose is removed from the blood stream as rapidly as it enters. This point is usually reached while glucose is still being absorbed from the intestine.

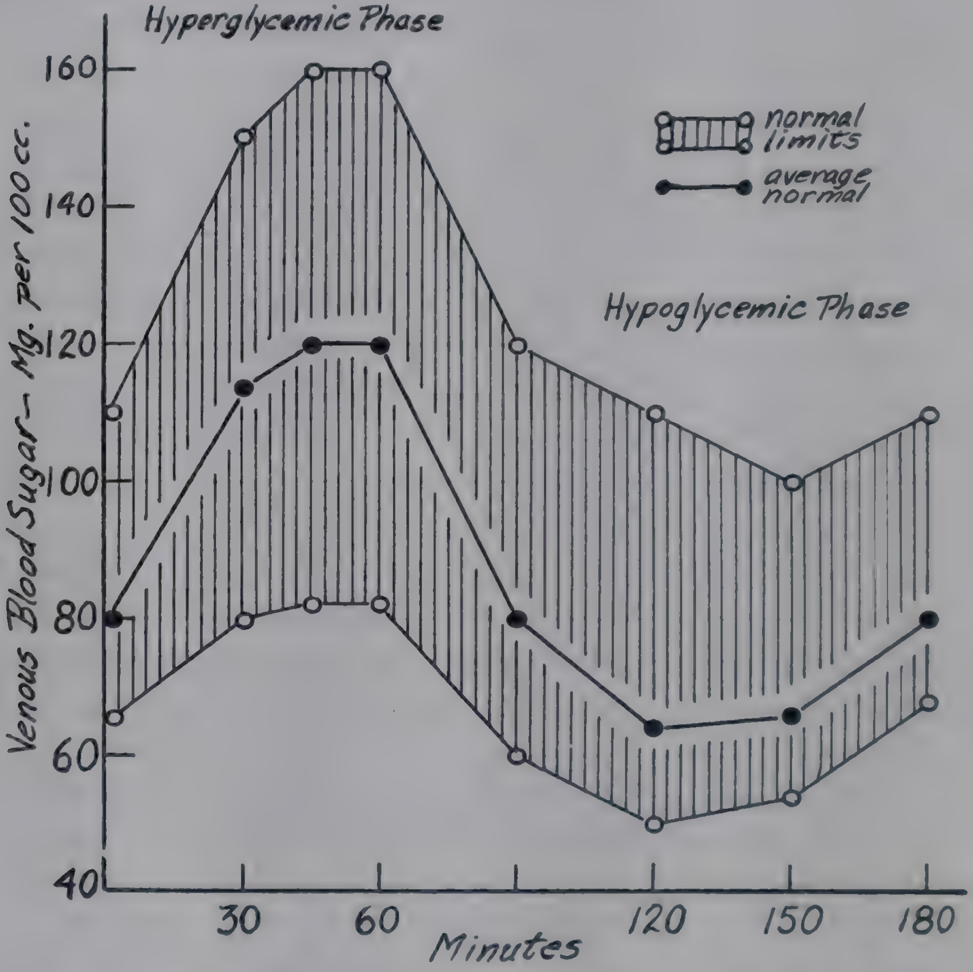


Fig. 6. Venous blood sugar curve after ingestion of 100 gm. glucose.

(*b*) A sharp fall to approximately the fasting level at the end of 1½ to 2 hours. Glucose is now leaving the blood faster than it is entering. This is due to continuing stimulation of the mechanisms indicated above (increased utilization and hepatic glycogenesis) and to slowing or completion of glucose absorption from the bowel.

(*c*) Continued fall to a slightly subfasting (10–15 mg. lower) concentration (hypoglycemic phase) and subsequent rise to the fasting level at 2½ to 3 hours. This hypoglycemic phase of the curve is due to the inertia of the regulatory mechanism. The decreased output of glucose by the liver and the increased utilization, induced by the rising blood sugar, are not reversed as rapidly as the blood sugar falls. The higher the initial rise, e.g., after intravenous injection of glucose, the more pronounced the hypoglycemic phase.

The changes in arterial (capillary) blood differ from those in venous blood characteristically as follows:

(*a*) The rise begins somewhat earlier. When the blood sugar has increased about 20 mg./100 cc., the rise in venous blood begins to lag behind that in arterial blood.

(*b*) At the peak, usually reached at 30 to 45 minutes, the level in arterial blood (150–220 mg./100 cc.) may be 20 to 70 mg. (average 30) higher than in venous blood. This arterial-venous blood sugar difference is an expression of the extent of removal of glucose from arterial blood by the tissues, i.e., of glucose utilization.

(*c*) The return to the fasting level (at 1½–3 hrs.) is not as rapid as in the case of venous blood, indicating that active removal from the blood, i.e., increased utilization, continues beyond the period of hyperglycemia. The two curves converge at the resting level or slightly below in 2½ to 3 hours.

Intravenous Glucose Tolerance Test. The possibility of abnormalities in the glucose tolerance curve due to abnormalities of absorption from the intestine may be obviated by injecting the glucose intravenously. In normal subjects, the nature of the curve depends upon the amount of glucose injected and the duration of the period of injection. However, when relatively small amounts are injected continuously at a constant rate, the blood sugar rises to a peak and then falls steadily during the period of administration, even at times to hypoglycemic levels. A satisfactory procedure consists in the intravenous injection of 0.5 gm. of glucose per kilogram of body weight in 20 per cent solution in distilled water by constant infusion over a period of thirty minutes. Blood is withdrawn for glucose determination before the injection is begun (control specimen), at the termination of the injection (30-minute specimen), thirty minutes later (1-hour specimen) and at subsequent hourly intervals for periods of two to six hours, as desired.

The blood sugar rises in normal subjects to a maximum of about 200 to 250 mg. per 100 cc. at the end of the injection, falls steadily to a slightly subresting level at two hours and returns to the pre-injection level at three to four hours, as in the case of the oral test. The hypoglycemic phase of the curve is obtained more consistently after intravenous than after oral administration of glucose, rendering this procedure of particular value in the study of conditions accompanied by hypoglycemia or increased glucose tolerance (p. 48).

Mechanism of Production of the Normal Glucose Tolerance Curve.

RISE OF BLOOD SUGAR. The initial rise in both arterial and venous blood sugar in the oral test is due in large measure directly to the glucose absorbed from the intestine which exceeds the capacity of the liver for removing it from the portal blood and passes into the systemic circulation.

ARTERIAL-VENOUS DIFFERENCE. This is an expression of the rate of removal of glucose from arterial blood by the tissues, particularly the muscles, for the formation of glycogen and for oxidation. Insulin plays an important part in these processes.

Fall in Blood Sugar. This appears to be due largely to three factors:

(*a*) *Removal of Sugar by the Liver to Form Glycogen.*

(*b*) *Removal of Sugar by the Extrahepatic Tissues for Glycogen Formation and Oxidation.* It has been generally believed that the hyperglycemia caused by the administration of glucose stimulates the pancreas to secrete more insulin, the resulting increased storage and utilization of glucose causing the fall in blood sugar following the primary rise. The validity of this hypothesis has been questioned in recent years and evidence now available suggests that the homeostatic mechanism in the liver is of greater fundamental importance than the pancreas in this connection.

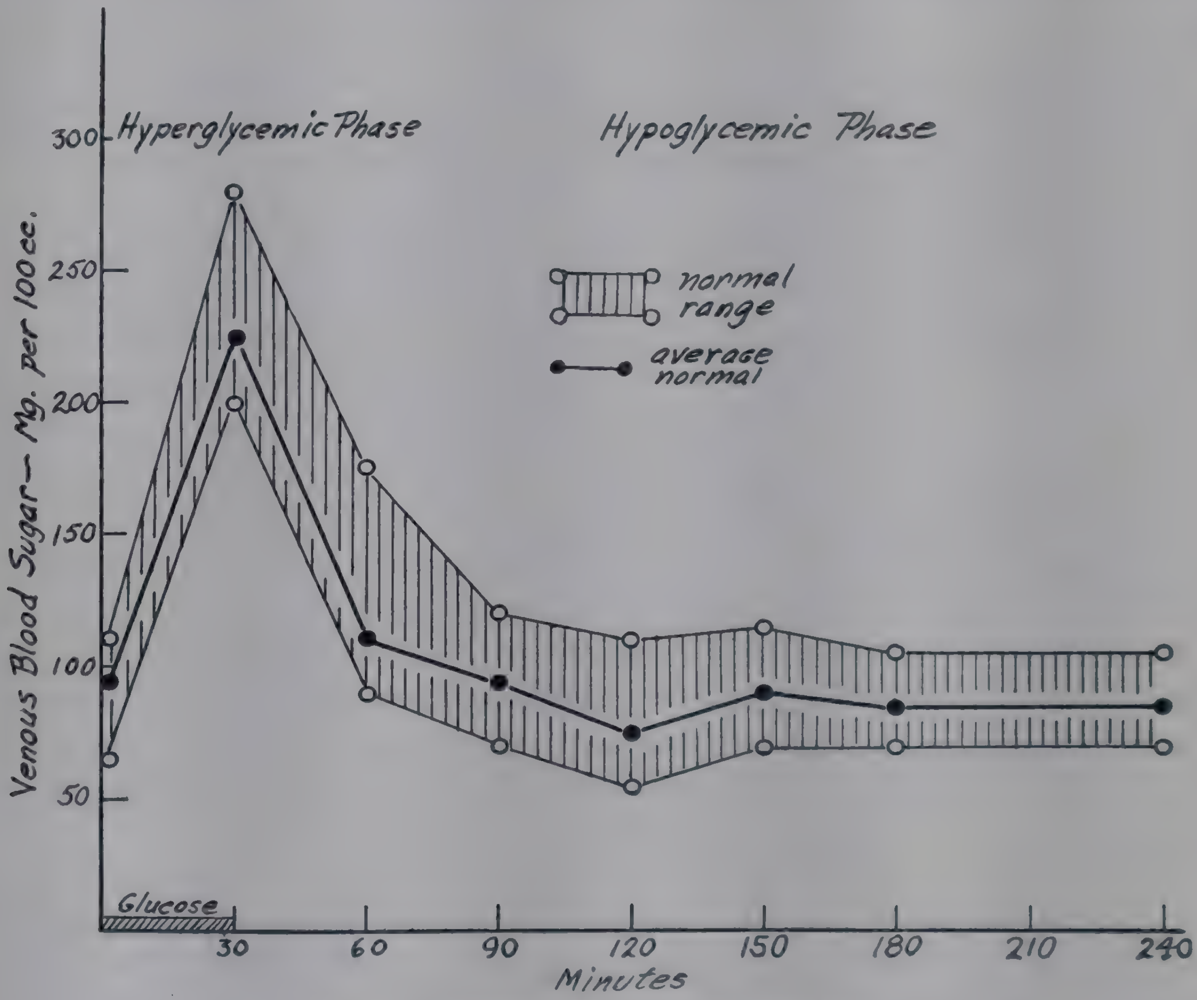


Fig. 7. Intravenous glucose tolerance test.

(*c*) *Diminished Hepatic Glycogenolysis and Gluconeogenesis.* The evidence referred to indicates that under normal conditions the liver responds to an increased blood sugar level by decreasing the output of blood sugar which it has been supplying from its own sources. An apt analogy has been drawn between this regulatory mechanism and a thermostatically controlled heating system. The liver corresponds to the furnace, the blood stream to the room, the tissues to the walls and the blood sugar concentration to the room temperature; the fuel is liver glycogen and the thermostatic regulation (i.e., of hepatic glycogenolysis) is represented by the interaction of hormones of the pancreatic islets (insulin), adrenal cortex, anterior hypophysis, and, under certain circumstances, also the thyroid, adrenal medulla (epinephrine) and perhaps the gonads; radiation of heat from the room is represented by the removal of glucose from the blood for oxidation and storage as muscle glycogen. Under normal conditions, as the blood sugar (i.e., room temperature) increases above the normal resting level (e.g., 100 mg./100 cc.), hepatic glycogenolysis and gluconeogenesis (i.e., heat production by the furnace) automatically diminish, and removal of sugar for storage and oxidation in the tissues (i.e., radiation of heat) is accelerated. Decrease in the blood sugar is automatically followed by increased glucose production in the liver and decreased rate of removal by the tissues. As stated by Soskin, the glucose tolerance curve, with its hyperglycemic and hypoglycemic phases, resembles the fluctuations in temperature above and below the threshold of regulation when extra heat is introduced into the system. The nature of the curve depends upon (1) the quantity of heat (amount of sugar) introduced, (2) the sensitivity and setting of the thermostat (endocrine balance) and (3) the capacity of the furnace and the fuel supply (ability of the liver to produce sugar and the glycogen store in the liver).

Factors Influencing Absorptive Blood Sugar Response. In normal subjects, the height to which the blood sugar rises increases with increasing doses of glucose up to about 50 gm., which produces a curve approximating that described above. The blood sugar rises no higher (in normal subjects) following ingestion of increased amounts of glucose (to 200 gm. or more), but the fall may be somewhat delayed. This is due to the fact that absorption of glucose from the intestine proceeds at a relatively constant rate regardless, within wide limits, of the quantity present. As soon as the increasing rate of utilization matches the increased rate of entrance of glucose into the blood stream, no further increment occurs in blood sugar concentration regardless of continuing absorption from the bowel. This is not the case in the presence of defects in the blood sugar regulating mechanism, e.g., in diabetes mellitus, in which the degree of elevation of blood sugar increases with increasing doses of glucose.

The glucose tolerance tends to be somewhat lower (i.e., the curve of blood sugar is higher and more prolonged) in old than in young subjects. Emotional disturbances, which tend to raise the blood sugar, may cause undue and abnormally prolonged hyperglycemia. Strenuous exercise before the ingestion of glucose may cause the blood sugar to rise to an abnormally high level, whereas strenuous exercise after ingesting the glucose may cause an abnormally marked and prolonged hypoglycemic phase in some subjects. Because of the several factors, physical, emotional, dietary and gastrointestinal, that may influence the curve, the conditions under which the test is performed should be rigidly standardized. It should be performed in the morning, at the time of the usual breakfast, the previous diet should be unrestricted (except in frank diabetes, when the test is usually superfluous) and, except when interest is centered particularly upon the hypoglycemic phase of the curve, physical exertion and emotional excitement should be avoided during the test period. Glucose tolerance tests must often be repeated before apparently abnormal results can be interpreted satisfactorily. Moreover, false normal or borderline normal curves are obtained at times as a result of disturbances of gastric emptying and intestinal motility and absorption. In such cases the intravenous test should be employed.

The state of carbohydrate nutrition influences the alimentary glucose response of normal individuals. An adequate deposit of glycogen in the liver and other tissues is essential to the production of a normal response as above described. If the subject is in a state of relative carbohydrate starvation the rise in blood sugar following the ingestion of glucose will be more pronounced and its fall more delayed than under normal conditions. Conversely, if the organism is in a state of carbohydrate saturation, i.e., if a high carbohydrate meal has been taken within two to three hours of the performance of the test, the rise in blood sugar will be distinctly less marked than that described above.

It is therefore important that the subject shall have partaken of a well rounded diet for some days prior to the performance of the test (Fig. 8). A diet high in fat and low in carbohydrate may result in a curve typical of a state of carbohydrate starvation. It is also well known that the repeated administration of glucose to normal persons at intervals of a few hours is followed by progressive lowering of the curve of alimentary hyperglycemia, i.e., a progressive increase in tolerance. These observations have been generally interpreted as indicating that the rather continuous absorption of adequate amounts of glucose from the intestine maintains active secretion of insulin by the pancreas and renders the islet cells sensitive to the stimulus to increased secretion furnished by the administration of glucose in the performance of the glucose tolerance test. When carbohydrates are withdrawn from the diet, insulin secretion is diminished, the machine idles, so to speak, and the sensitivity of the islet cells to sudden stimulation is diminished. When glucose is administered subsequently, an adequate amount of insulin is not secreted promptly and removal of the absorbed glucose from the blood stream is delayed. The relatively slight hyperglycemic effect of repeated doses of glucose is generally attributed to a state of increased responsiveness on the part of the pan-

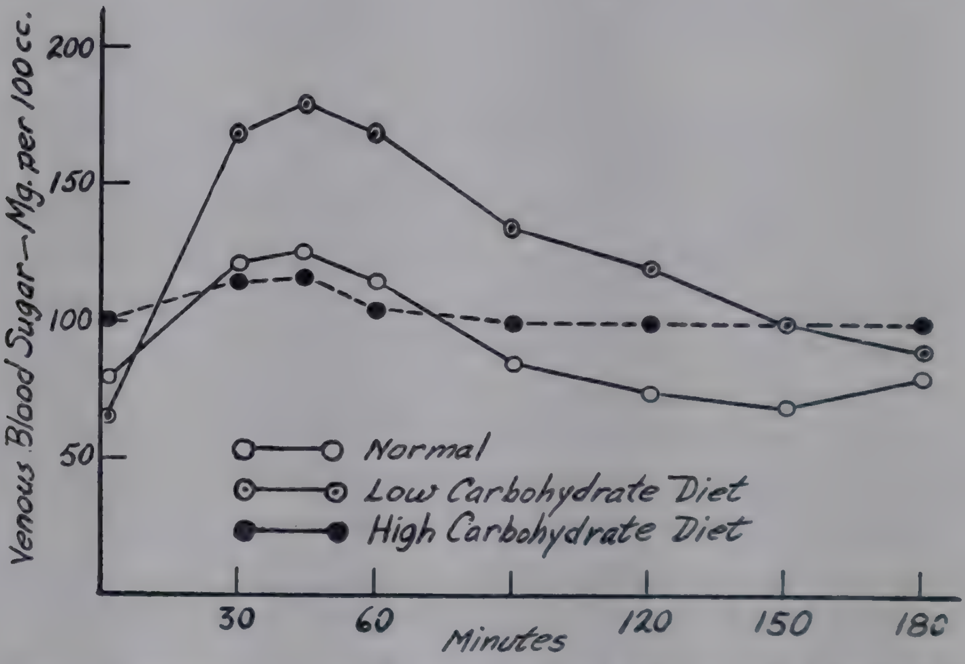


Fig. 8. Effect of previously high and low carbohydrate diets on oral glucose tolerance test.

creas, resulting from the previous administration of glucose. This influence of previous sugar administration on subsequent dextrose tolerance curves is known as the Staub-Traugott or Hamman-Hirschman effect.

It has also been suggested that the decreased blood sugar response that follows the repeated administration of glucose is due to a decreased output of glucose from the liver (decreased hepatic glycogenolysis) and not to increased secretion of insulin, although these two phenomena are not necessarily mutually exclusive (p. 14).

The One-Hour, Two-Dose Glucose Tolerance Test. The Staub-Traugott phenomenon, referred to above, may be described briefly as follows: Normal human subjects react to repeated doses of glucose with either diminution or little or no change in glycemia, while diabetics react with definite hyperglycemia. This phenomenon has been utilized as the basis for several modifications of the glucose tolerance test. One of the most widely employed of these is the one-hour, two-dose test proposed by Exton. The test is performed as follows: Dissolve 100 gm. of glucose in about 650 cc. of water. This solution is then flavored with lemon juice and divided into two equal doses, each containing 50 gm. of glucose in about 15 per cent solution. In the morning after a twelve-hour fast collect blood and urine samples and give the first dose of glucose, allowing one-half minute for its ingestion. Thirty minutes later collect a second blood sample and give the second dose of glucose. Thirty minutes after the ingestion of the second dose of glucose collect blood and urine samples.

Normal criteria are: (1) Normal fasting blood sugar; (2) a half-hour level less than 50 mg. above the fasting value; (3) a one-hour value less than 30 mg. above the half-hour value. In our experience, however, the following criteria are the most acceptable: (1) Normal fasting blood sugar; (2) one-hour blood sugar less than 160 mg. per 100 cc.; (3) all urine specimens sugar-free.

The use of this procedure is practically restricted to the diagnosis of diabetes mellitus and, in our experience, has not been as satisfactory for routine investigation of glucose tolerance as the longer oral and intravenous tests.

EFFECT OF OTHER SUGARS

The metabolism of fructose differs from that of glucose. The difference between the behavior of these two sugars may be illustrated by certain experimental observations.

(*a*) Liver and muscle contain separate hexokinases for glucose and fructose. Insulin (in the presence of growth hormone and adrenocortical hormones) is necessary for normal activity of the glucokinase ("hexokinase") (p. 15) but not the fructokinase reaction. Consequently, in the absence of insulin, phosphorylation of and, therefore, glycogenesis from fructose may be uninhibited, whereas phosphorylation of and glycogenesis from glucose is impaired considerably.

(*b*) Insulin hypoglycemia is relieved by glucose more effectively than by fructose.

(*c*) Fructose produces the same rise in blood pyruvic acid and fall in inorganic phosphate in diabetic as in normal subjects, whereas glucose does not.

(*d*) Following the ingestion of fructose there is comparatively little rise in blood sugar and practically no arterial-venous difference, indicating the minor role of the muscles in removing this sugar from the blood.

(*e*) Inasmuch as insulin is not necessary for essentially normal glycogenesis from and oxidation of fructose, this sugar has frequently been advocated as a substitute for glucose in the treatment of diabetes mellitus. However, a portion of the fructose is converted to glucose in the liver; the subsequent utilization of this fraction depends upon the availability of insulin. Moreover, this hormone is necessary for normal lipogenesis from fructose, as from glucose. In view of the fact that about 30 per cent of the daily carbohydrate intake is converted to fat, impairment of this major pathway of utilization probably constitutes an additional reason why fructose has little if any practical advantage over glucose in the management of clinical diabetes.

(*f*) Cori has found that although fructose is absorbed from the intestine much more slowly than glucose, at the end of four hours the amount of glycogen formed in the liver by the former was 39 per cent and by the latter 17 per cent. In the absence of hepatic disease or functional insufficiency the ingestion of fructose, in tolerance doses, is followed by comparatively little elevation of blood sugar, since, after absorption, it is effectively removed from circulation by the liver where it is stored as glycogen. The fructose tolerance test has therefore been utilized as a test of the integrity of liver function. It is performed as follows:

A sample of blood is taken in the fasting state. Fifty grams of fructose (glucose-free) are ingested, dissolved in 200 cc. of water or lemonade. Samples of blood are withdrawn at one-half-hour intervals over a period of two hours and the sugar content of all specimens determined. A normal response is characterized by:

(1) A maximum rise of less than 30 mg. per 100 cc. above the post-absorptive level.

(2) A return to the resting level in one and one-half hours.

This procedure is probably improved if the blood fructose concentration alone is determined rather than fructose plus glucose, as above. Normally, the blood fructose concentration, 0 to 8 mg. per 100 cc. in the fasting state, increases not more than 15 mg. per 100 cc., usually within the first hour after ingestion of 50 gm. of fructose, and falls to 0 to 10 mg. per 100 cc. at the end of two hours.

Galactose Tolerance. Galactose, like fructose, is metabolized chiefly by the liver. The metabolism of galactose differs from that of glucose chiefly in that (*a*) it is absorbed from the intestine somewhat more rapidly than the latter, (*b*) the magnitude of its increase in the blood after ingestion is much smaller than in the case of glucose, (*c*) the "renal threshold" for excretion of galactose is very low and (*d*) its intermediary metabolism is apparently influenced much less than that of glucose by the function of organs other than the liver.

After ingestion of 40 gm. of galactose, the blood galactose concentration normally reaches a maximum of 15 to 35 mg. per 100 cc. in thirty to sixty minutes, falling to zero at the end of two hours. Normal subjects may excrete up to 3 gm. of galactose (or galactose plus glucose) in the urine during the five hours immediately following ingestion of 40 gm. of this sugar. Intravenous injection of

1 cc. of a 50 per cent solution of galactose per kilogram of body weight is followed by its rapid disappearance from the blood stream in normal subjects, none remaining at the end of 75 minutes.

Insulin Tolerance Test

The purpose of the insulin tolerance test is to determine (*a*) the sensitivity of the organism to insulin and (*b*) its responsiveness to insulin-induced hypoglycemia. Under normal conditions, the blood sugar falls to about 50 per cent of the fasting level twenty to thirty minutes after intravenous injection of 0.1 unit of insulin per kilogram of body weight. It then rises rapidly, reaching the pre-injection level in ninety to one hundred and twenty minutes (Fig. 12, p. 53). The duration of the hypoglycemia is of greater significance than its degree. The precautions that must be observed in performing this test are the same as those described in connection with the glucose tolerance test, since the response in both instances is influenced by the same factors. In cases in which an exaggerated or prolonged hypoglycemic response is anticipated (p. 48), it is advisable to provide for prompt administration of glucose as soon as clinical manifestations of hypoglycemia develop and, at times, to use $\frac{1}{2}$–$\frac{1}{3}$ the standard dose of insulin. In such cases it is also advisable to administer carbohydrate about three hours after the test is completed.

Insulin-Glucose Tolerance Test

This procedure was devised as a substitute for the conventional insulin tolerance test. It involves less risk than the latter and is regarded as a more sensitive means of detecting hypoglycemia unresponsiveness.

After an overnight fast, a sample of blood is taken and 0.1 unit of regular insulin per kg. is injected intravenously. After 30 minutes, or when the first significant manifestation of hypoglycemia develops, whichever is first, 0.8 gm. of glucose per kg. is given by mouth. Samples of blood are taken at 30, 60, 90, 120, and 180 minutes after the injection of insulin.

In normal subjects, the rise in blood sugar following ingestion of glucose in the hypoglycemic state exceeds that which follows administration of glucose without insulin. Representative normal values for blood sugar with this procedure are: 30 minutes, 30 to 55 mg. (40); 60 minutes, 100 to 135 mg. (115); 90 minutes, 125 to 175 mg. (150); 120 minutes, 105 to 165 mg. (140); 180 minutes, 75 to 115 mg. (95).

Epinephrine Tolerance Test

The increase in blood sugar which follows administration of epinephrine has been utilized as an index of the quantity and availability of glycogen in the liver. After intramuscular injection of 1 cc. of a 1:1000 solution of epinephrine hydrochloride, the blood sugar concentration normally increases 35 to 45 mg. per 100 cc. in forty to sixty minutes, returning to the resting level in 1$\frac{3}{4}$ to 2 hours.

Phenomena Associated with Normal Alimentary Glucose Reaction

The increased utilization of glucose which follows its absorption from the intestine in normal subjects is accompanied by other biochemical changes.

Decreased Serum Phosphate. The inorganic phosphate of the blood plasma appears to be intimately related to the intermediary metabolism of glucose. Formation of hexose phosphates occurs in the process of utilization of glucose. Consequently, during this process inorganic phosphate is withdrawn from the plasma; the phosphate content of the tissues, particularly muscle, increases and phosphate excretion decreases. These changes in phosphate occur independently of the level of blood sugar; e.g., they continue beyond the period of hyperglycemia induced by glucose administration and occur also during the hypoglycemia induced by administration of insulin. The hypophosphatemia is therefore a reflection of the increased utilization of glucose.

Decreased Serum Potassium. Stimulation of glucose utilization is accompanied by a shift of potassium from extra to intracellular fluids. This occurs most strikingly when glucose and insulin are administered after a period of carbohydrate restriction or to subjects with uncontrolled diabetes mellitus. This shift may be sufficiently pronounced to produce a fall in the serum potassium concentration. The basis for this phenomenon is not clear, but it may be related to the passage of phosphate ion in the same direction, i.e., to the increased utilization of glucose.

Increased Respiratory Quotient. An increase in R.Q. above the normal resting level (0.82) is usually regarded as an indication of increased carbohydrate utilization (oxidation, lipogenesis). About 1 to 1½ hours after oral administration of glucose (100 gm.) the R.Q. rises from 0.82 to 0.88–0.90, reaching 0.95 or 0.96 in about 2 hours and then gradually falling to the resting level in about 4 hours. The period of this increase coincides approximately with that of hypophosphatemia, discussed above.

ABNORMALITIES OF POSTABSORPTIVE BLOOD SUGAR LEVEL

Fasting Hyperglycemia

Fasting hyperglycemia may be due to either an increased rate of entrance of glucose into the blood from the liver (increased hepatic glycogenolysis or gluconeogenesis) or a decreased rate of removal of glucose from the blood by the tissues (decreased storage and utilization). In many clinical disorders both of these mechanisms are operative and it seems probable that persistent hyperglycemia may be dependent fundamentally upon elevation of the threshold of sensitivity of the hepatic glycogenolytic mechanism to the blood sugar, i.e., a disturbance of the homeostatic mechanism in the liver (p. 22).

Diabetes Mellitus. The fundamental fault in diabetes mellitus is apparently a deficiency in pancreatic islet secretion (insulin). Demonstration of the influence of hormones of the anterior hypophysis and adrenal cortex upon carbohydrate metabolism (p. 16) has raised some doubt as to whether a quantitative deficiency in insulin secretion is invariably the only cause of diabetes mellitus (p. 68). The exact way in which insulin acts in the intermediary metabolism of carbohydrates is not definitely understood but it appears to increase the utilization of glucose in the tissues, increase glycogenesis, and also probably retards glycogenolysis in the liver. In the absence of an adequate amount of insulin, hepatic glycogenolysis and gluconeogenesis are increased and the rate of removal of glucose from the blood for glycogenesis in the extrahepatic tissues is decreased. The obvious consequence is an increase in the concentration of glucose in the blood. The utilization of glucose at ordinary levels of blood sugar is probably impaired, but there is evidence that at very high blood sugar levels glucose can be utilized by the tissues in the presence of insulin deficiency and even in the absence of insulin if the anterior hypophysis or adrenal cortex has been removed. These facts suggest that the fundamental mechanism underlying the hyperglycemia of diabetes mellitus involves overproduction (excessive hepatic glycogenolysis and gluconeogenesis) as well as underutilization of glucose. On the basis of the homeostatic mechanism in the liver, one may assume that because of the disturbed endocrine balance (diminished insulin, with relative excess of anterior pituitary and adrenal cortex), the "thermostat" regulating the mechanism has been set at a higher level, allowing the blood sugar to rise to a hyperglycemic level before hepatic glycogenolysis and gluconeogenesis are depressed.

In large series of cases of diabetes mellitus the postabsorptive blood sugar has ranged from 70 to 1850 mg. per 100 cc. of blood. Complicating conditions, such as acidosis, which should be considered a part of the disease, and hyperthyroidism, tend to maintain the blood sugar at a higher level than that due to the insulin deficiency per se. Although it is true that the level of the fasting blood sugar usually parallels the severity of the condition, such a statement cannot be made unequivocally. In early cases the fasting blood sugar may be well within normal limits; as the condition becomes more advanced values of 180 to 300 mg. may be obtained; in advanced cases values of 400 mg. are not uncommon and, if acidosis is marked and the patient in coma, the degree of hyperglycemia may be extreme (700 mg. or over). Figures above 600 mg. are, however, rarely observed.

In some instances the postabsorptive blood sugar concentration does not afford a true index of the presence or severity of an existing diabetes. As has been stated, in mild cases the fasting blood sugar may be within normal limits and further studies, such as the glucose tolerance test, must be resorted to in order to establish the true nature of the condition. This will be discussed later in greater detail (p. 43). Fasting hyperglycemia (above 130 mg. per 100 cc.) is highly suggestive of diabetes mellitus, but other conditions must be considered. In the presence of complicating factors, particularly hyperthyroidism, the diagnostic standard must be raised, according to some authorities, to a blood sugar of 150 mg. per cent fasting or 200 mg. per cent or more after meals, in addition to glycosuria.

The blood glucose is derived from hepatic glycogen in the absence of an exogenous supply. Removal of the liver in complete diabetic (depancreatized) animals results in the disappearance of sugar from the blood as in the case of nondiabetic animals. Advanced acute hepatic disease with serious disturbance of the glycogenic function of the liver, occurring in a patient with diabetes, may tend to diminish the blood sugar concentration (phosphorus or arsenic poisoning, toxemias of pregnancy, acute diffuse necrosis of liver, cirrhosis, and occasionally acute catarrhal jaundice). It is interesting to note in this connection that several observers have called attention to the occurrence of hypoglycemic reactions in patients with hemochromatosis receiving insulin, and to the marked fluctuation in glucose tolerance in that condition. This peculiar state is somewhat comparable to that of the hypophysectomized-depancreatized dog, in which the blood sugar rises higher after glucose than in the pancreatectomized dog and hypoglycemic manifestations develop more rapidly and at a higher blood sugar level than in the hepatectomized animal. It must be emphasized that whereas the superimposition of hepatic disease occasionally is associated with a lowering of the fasting blood sugar concentration in diabetics, it usually further diminishes the already lowered glucose tolerance of these individuals and increases their resistance to insulin. A tendency toward lowering of the fasting blood sugar level occurs in undernourished individuals whose carbohydrate and protein intake has been so restricted that the available glycogen store in the liver has been depleted. Under these circumstances the postabsorptive blood sugar level may mask the severity of the diabetic condition. On the other hand, as has been indicated, hyperthyroidism, hypertension, nephritis, acidosis and acute infections, occurring in a patient with diabetes mellitus may, by their independent hyperglycemic effect, exaggerate the apparent severity of the condition. It should be realized, however, that the metabolic error in diabetes is seriously aggravated by factors which increase hepatic glycogenolysis, such as hyperthyroidism, infection and acidosis, and that their presence is of definitely adverse prognostic import.

Hyperthyroidism. Hyperthyroidism, whether due to thyroid disease (exophthalmic goiter, toxic adenoma) or temporarily induced by the administration of thyroid gland substance or thyroxine, results in a state of hypersensitiveness on the part of the liver for the conversion of glycogen into glucose. This glycogenolytic action of thyroxine may be excited directly, but it is more probable that the effect is produced by rendering the liver hypersensitive to sympathetic nerve impulses, and possibly to epinephrine, since section of the splanchnic nerves prevents the discharge of glycogen. Increased glycogenolysis and also gluconeogenesis perhaps result also from the increased rate of tissue metabolism. In the presence of an adequate supply of glycogen in the liver there is a tendency toward a state of fasting hyperglycemia, the tissues being constantly subjected to the necessity of handling a superabundance of glucose. This, unless excessive, is compensated by increased activity of the storage mechanism and increased combustion of glucose in the tissues. Obviously, unless carbohydrates are supplied in abundance, the store of hepatic glycogen will be depleted and the blood sugar will automatically fall and may become subnormal. This perhaps accounts for the relatively infrequent incidence of fasting hyperglycemia in patients with hyperthyroidism, which is rarely severe and which has been reported as occurring in 0.5 to 8.5 per cent of large series of cases.

The mistake should not be made of interpreting a disturbance of carbohydrate metabolism dependent upon hyperthyroidism as due to diabetes mellitus. Further details of the laboratory means of differentiating these conditions will be discussed later (pp. 43, 46, 71, 446). Patients with hyperthyroidism are undoubtedly more prone to develop diabetes than other individuals, the combination being encountered in about 1 to 3 per cent of patients with hyperthyroidism.

Increased Secretion of Epinephrine. The hepatic glycogenolytic action of epinephrine has been referred to previously. If the glycogen content of the liver is adequate, the injection of epinephrine is followed by a rise in blood sugar. This fact has been utilized as a test of the glycogen storing capacity of the liver. If 0.62 cc. of a 1:1000 solution of epinephrine hydrochloride is injected intramuscularly, the blood sugar rises 35 to 45 mg. per 100 cc. in $\frac{3}{4}$ to 1 hour and returns to the resting level in $1\frac{3}{4}$ to 2 hours.

This effect of epinephrine is probably involved in several physiologic and pathologic states.

(*a*) As indicated above, it appears to be a factor in the production of the hyperglycemic response to increased thyroid activity.

(*b*) Hypoglycemia, produced characteristically by the administration of insulin, results in increased epinephrine secretion with consequent mobilization of sugar from the liver. This arrangement represents another example of automatic adjustment when a disturbance threatens the equilibrium of the organism.

(*c*) Various emotional states such as anger, anxiety and fear are associated with an increased quantity of epinephrine in the blood. These states, as well as conditions of mental stress, excitement and excessive cold, have been found to be accompanied by an increase in blood sugar, apparently the result of the mobilization of liver glycogen by epinephrine. Pain and discomfort perhaps act in a similar manner. These conditions constitute what may be termed psychic hyperglycemia.

(*d*) Increased epinephrine secretion is a factor in the production of hyperglycemia in the so-called "diabetic piqûre" and in intracranial disorders (concussion, brain tumor, fracture of the skull and intracranial hemorrhage, with increased intracranial pressure) which cause the transmission of impulses through the splanchnic nerves to the adrenals and liver. It has also been observed in association with lesions in the region of the fourth ventricle and the hypothalamus, particularly the pons. Hyperglycemia may occur in a variety of convulsive states, including idiopathic epilepsy, Jacksonian epilepsy, tetanus, tetany, eclampsia and hypertensive encephalopathy, if the hepatic glycogen stores are adequate. If the latter are depleted, the blood sugar does not rise and indeed may fall if the convulsive state is prolonged.

(*e*) Fasting hyperglycemia, either constant or temporary and occurring periodically, has been observed in association with certain tumors of the adrenal medulla (pheochromocytomas), presumably dependent upon the periodic outpouring of excessive quantities of epinephrine into the blood stream. There is a rather characteristic clinical picture of paroxysmal hypertension with simultaneous paroxysms of hyperglycemia. The blood sugar concentration in the intervening periods may be normal and the hyperglycemia (and hypertension) tends to be inhibited by ergotamine and benzodioxane compounds, which counteract the effect of epinephrine. In certain cases, hyperglycemia and hypertension are persistent rather than paroxysmal.

Adrenal Cortical Hyperfunction. This condition, resulting from hyperplasia or tumor of the adrenal cortex, may result in (*a*) the adrenogenital syndrome or (*b*) Cushing's syndrome. The former is seldom accompanied by abnormality in carbohydrate metabolism; the latter is usually characterized by the gradual development of diminished glucose tolerance, followed by progressive elevation of the fasting blood sugar concentration, which may, however, remain normal for a long time. The hyperglycemia, when it develops, is due to increased hepatic gluconeogenesis and decreased glucose utilization (p. 16). This so-called "steroid diabetes" is characterized by resistance to insulin, mildness during fasting, and negative nitrogen balance in the absence of marked glycosuria.

Hyperpituitarism. The hyperglycemic effect of anterior pituitary extracts has been considered previously (p. 16). Two clinical hyperpituitary states may at times be accompanied by hyperglycemia, i.e., acromegaly (eosinophilic adenoma) and the type of Cushing's syndrome due to pituitary basophilism or basophilic adenoma. The fasting blood sugar is usually normal in patients with acromegaly, particularly in the early stages of the disease, before the tumor has encroached extensively upon the remainder of the gland. In a large series of cases, about 10 per cent have moderate and 10 to 15 per cent mild fasting hyperglycemia, but many more have a diminished glucose tolerance. Diabetes mellitus occurs in about 15 to 20 per cent of acromegalics, the former condition developing within fifteen years of the onset of acromegaly in the majority of instances (average 9.5 years). In several instances we have observed a fall in a previously elevated blood sugar, with improvement in glucose tolerance, as the pituitary tumor increased in size.

Cushing's syndrome due to pituitary basophilism may be accompanied by manifestations of disturbed carbohydrate metabolism described in connection with adrenal cortical hyperfunction. These phenomena are dependent largely upon excessive adrenocorticotrophic stimulation of the adrenal cortex. The effect may in part, however, be exerted by an anterior pituitary hormone acting in some manner other than through the adrenal cortex (p. 16) (growth hormone).

Anesthesia, Asphyxia, Hypnosis. The exact cause of the acidosis of ether and chloroform anesthesia is not known but it is well established that the hydrogen ion concentration of the blood increases rather suddenly during the induction of anesthesia and then rises gradually during its maintenance. Although the ether and chloroform may exert a direct glycogenolytic influence upon liver glycogen, anoxia may play an important role in the production of this effect. A remarkable rise in blood sugar may occur during anesthesia. The degree of hyperglycemia is dependent upon the amount of glycogen in the liver, the quantity of anesthetic administered and upon the adequacy of the mechanism of carbohydrate utilization.

In the absence of any other factor which tends to produce hyperglycemia, such as hyperthyroidism or diabetes mellitus, the blood sugar has been found to rise 7 to 8 mg. per 100 cc. per ounce of ether administered. These figures may be greatly increased if the anesthetic is not skillfully administered, by the introduction of complicating factors such as excessive muscular effort, excitement and asphyxia. In diabetes mellitus the degree of hyperglycemia is much more marked as the excessive amount of glucose entering the blood stream cannot be stored or utilized adequately by the tissues. The duration of anesthesia hyperglycemia, usually four to twelve hours after operation, depends upon the quantity of anesthetic used and the duration of the state of anesthesia. The rise appears to be more pronounced in laparotomies than in extra-abdominal operations but is otherwise independent of the nature or severity of the operative procedure. Values as high as 400 mg. per 100 cc. have been observed in individuals with normal carbohydrate metabolism.

If the quantity of glycogen stored in the liver is deficient, as in severe hepatocellular damage, the blood sugar cannot rise as in normal individuals and there is great danger of depletion of the hepatic glycogen reserve with consequent serious damage to the liver by the ether or chloroform which have a profound toxic effect upon the liver parenchyma. It has also been found that practically all of the barbituric acid series of hypnotics cause a rise in blood sugar and a simultaneous fall in the glycogen content of the liver. The effects of these various anesthetic agents may be attributable in part to asphyxia, to a direct action of the anesthetic on the liver, or to liberation of epinephrine from the adrenal glands.

Anoxemia, whether due to mechanical causes or occurring in carbon monoxide poisoning or heart failure or during anesthesia, particularly with nitrous oxide and ethylene, is accompanied by an increase in blood sugar. The two anesthetic agents mentioned do not, in the absence of asphyxia, have any appreciable effect upon the blood sugar concentration. Anoxemia produces this effect probably by increasing mobilization of hepatic glycogen. The blood sugar may rise 20 to 40 mg. per 100 cc. after the administration of morphine, the mechanism probably being similar to that involved in asphyxia. Transient hyperglycemia and glycosuria have been reported in patients with acute coronary artery occlusion. The mechanism operating here may be due to sudden circulatory inefficiency but may be contributed to by the pain and anxiety incident to the acute attack.

Acidosis. Acidosis diminishes the blood sugar lowering action of insulin. Although the mechanism involved is not completely understood, it appears that in acidosis there is interference with the phosphorylation of glucose, a process that is fundamentally involved in the cellular uptake of this substance (hexokinase reaction, p. 5). There is apparently little or no interference with the phosphorylation of fructose in acidosis.

This factor is of particular importance in diabetes mellitus because it aggravates a pre-existing hyperglycemic mechanism, in this case hypoinsulinism. In the evaluation of the significance of the fasting blood sugar concentration in this disorder it is important to attempt to determine what proportion of the elevation is dependent upon the complicating state of acidosis (ketosis). Acidosis due to other causes, as fever, nephritis and dehydration, may result in an increase in the level of blood sugar. The rise in such cases is not marked, but, occurring in association with other factors, its influence may be significant.

Hepatic Disease. Although diminished glucose tolerance (p. 45) and a normal or slightly subnormal fasting blood sugar level (p. 40) occur much more commonly than fasting hyperglycemia in patients with hepatocellular damage, the latter is observed occasionally. Fasting values as high as 185 mg. per 100 cc. have been reported in patients with acute hepatitis in the absence of true diabetes mellitus. This is apparently the result of excessive glycogenolysis. In some cases, hyperglycemia in patients with hepatic or biliary tract disease may be due to associated pancreatitis, but this in itself seldom causes hyperglycemia. Fasting hyperglycemia, often marked, is a common but not invariable feature of hemochromatosis (p. 261), being due fundamentally to involvement of the islands of Langerhans in the fibrotic and degenerative process in the pancreas.

Miscellaneous. Strenuous muscular exercise causes liberation of glucose from the liver with resulting hyperglycemia. It must be realized that, as in all conditions acting through the agency of increased hepatic glycogenolysis, hyperglycemia can only be produced if the reserve supply of liver glycogen is abundant. When this is depleted, the blood sugar concentration decreases and, eventually, hypoglycemia may result. This fact is perhaps responsible for the contradictory reports of the blood sugar level in eclampsia and other convulsive states. Similarly, strenuous muscular exertion, in a normal individual, is at first associated with an increase and later, if continued, with a decrease in blood sugar, the source of supply having been virtually exhausted.

Fasting hyperglycemia may occur in many acute and chronic infections although usually the fasting blood sugar is within normal limits and the tolerance for glucose is lowered (p. 48). This is believed to be due to increased and prolonged hepatic glycogenolysis and gluconeogenesis, which may seriously aggravate a condition of latent or frank diabetes mellitus. A mild degree of hyperglycemia has been observed in about 5 to 40 per cent of cases of essential hypertension and occasionally in nephritic hypertension. Its incidence in the former condition is highest in patients with obesity, a condition that is frequently accompanied by a lowered tolerance for glucose (p. 48). Such patients (obesity and hypertension) may in reality be potential diabetics.

Moderate elevations of blood sugar may occur in acute and chronic pancreatitis, but this is by no means a constant finding. An increase occurs as a result of smoking, due probably to the epinephrine-stimulating effect of nicotine. It may occur also after administration of caffeine, quinine, pituitrin and Benzedrine (amphetamine), and has been reported in methyl salicylate poisoning.

Fasting Hypoglycemia

Postabsorptive hypoglycemia is encountered much less frequently than hyperglycemia. However, with the development of more specific knowledge regarding the influence of the liver and pancreas upon carbohydrate metabolism, hypoglycemia has achieved a clinical significance which was formerly lacking. The factors which tend to lower blood sugar are the opposite of those which have been dealt with in the discussion of hyperglycemia: (*a*) decreased rate of entrance of glucose into the blood (decreased hepatic glycogenolysis and gluconeogenesis);

(*b*) increased rate of removal of glucose from the blood (increased storage or utilization in the extrahepatic tissues). The latter seldom produces fasting hypoglycemia by itself, except under unusual conditions of severe and prolonged muscular activity (marathon runners), but a tendency toward hypoglycemia due to other causes may be aggravated by exercise.

Hyperinsulinism. Following injection of insulin the blood sugar concentration falls, usually reaching a minimum in two to four hours, the degree of reduction and duration of hypoglycemia depending upon the amount of insulin administered. After protamine zinc insulin, the blood sugar usually reaches a minimum in 12 to 16 hours, the effect lasting for about 24 hours. Symptoms of hypoglycemia usually occur when the blood sugar level reaches 45 mg. per 100 cc. (Folin-Wu) although some patients apparently have an extraordinary tolerance for low levels of blood sugar. Cases of diabetes have been reported with blood sugar values ranging from 23 to 31 mg. per 100 cc. over a period of six hours without symptomatic manifestations. Since values below 25 mg. (Folin-Wu) probably represent nonglucose reducing substances, sugar was practically absent from the blood stream at these levels. The true blood sugar is practically zero at the time of occurrence of hypoglycemic convulsions in rabbits. It is obvious that hypoglycemia of any degree can be induced by the injection of insulin, the responsiveness of different individuals varying within rather wide limits.

Apart from this form of induced hyperinsulinism, a similar mechanism may be brought into play by other means. In normal individuals, 2½ to 3½ hours after the administration of carbohydrate or epinephrine there is a period of mild hypoglycemia (50–70 mg. per 100 cc.) following the primary rise in blood sugar. It has been assumed that the mechanism of glycogen storage, stimulated by the rise in blood sugar, acquires a momentum which carries the process beyond the period of increased glucose supply. However, there is some evidence that the hypoglycemic reaction following cessation of prolonged sugar administration to animals does not depend upon increased mobilization of insulin from the pancreas, since it may occur in depancreatized animals receiving a constant supply of insulin. The fall in blood sugar which follows the primary rise accompanying the absorption of ingested sugar may be due to a decrease in the supply of glucose by the liver to the blood, in response to the influx of exogenous sugar.

Spontaneous hyperinsulinism may occur as a result of hyperplasia, adenoma and, rarely, carcinoma of the islands of Langerhans of the pancreas. The fasting blood sugar may be as low as 20 to 50 mg. per 100 cc., but is not necessarily consistently subnormal. In such cases, hypoglycemia may be induced or accentuated by (*a*) moderate exercise after a prolonged fasting period or (*b*) performing an oral or, preferably, an intravenous glucose tolerance test, the hypoglycemic phase of the blood sugar curve being characteristically exaggerated and prolonged (p. 48). In establishing the diagnosis, significant hypoglycemia must be shown to be present at the time of occurrence of the characteristic subjective and objective manifestations, which must also be largely relieved promptly by intravenous injection of glucose. It must be borne in mind, however, that the occurrence or severity of symptoms bears no constant relation, even in the same subject, to the actual level of the blood sugar. The rapidity as well as the extent of the fall may be important in this connection. In cases of latent or mild hyperinsulinism, the condition may be evidenced only by an exaggerated response to insulin administration (insulin tolerance test, p. 53).

Hypoglycemia due to hyperinsulinism occurs at times in the newborn of mothers with uncontrolled diabetes. In some fatal cases no morphologic abnormalities have been observed in the pancreas, whereas in others there has been hyperplasia or hypertrophy of the islands of Langerhans. It is believed that during intra-uterine life prolonged exposure of the fetus to an abnormally high blood sugar concentration results in stimulation of excessive insulin secretion, with or without consequent hyperplasia of the islets. Following delivery, this increased insulin production often continues and exceeds the requirement of the infant, and hypoglycemia develops, frequently with serious consequences unless it is anticipated and treated promptly and adequately.

Hepatic Disease. Since the demonstration of the essential part played by the liver in the maintenance of the normal blood sugar level, several cases of hepatogenic hypoglycemia have been reported. The liver is endowed with such an extensive functional reserve capacity and with such remarkable regenerative powers that its glycogenic function is seriously impaired only in the late stages of chronic diseases such as cirrhosis. Blood sugar values of 50 to 60 mg. per 100 cc. may be observed, rarely, in the terminal stages of cirrhotic processes, particularly in obstructive biliary cirrhosis but at times also in the portal type. Hypoglycemia may be a terminal event after operations for biliary tract disease under general anesthesia which may exhaust a glycogen reserve already depleted by associated hepatic disease (hepatitis).

It is in the acute, rapidly progressive and extensive forms of hepatic disease that hypoglycemia is most commonly observed. Values as low as 25 to 40 mg. per 100 cc. have been reported in cases of phosphorus, benzol, chloroform and carbon tetrachloride poisoning and following administration of arsphenamine, synthalin and sulfonamides, usually, however, in terminal stages. Severe acute infections, such as diphtheria and scarlet fever, may produce similar grades of hypoglycemia, perhaps in the same manner, although suprarenal insufficiency may be a factor in such cases. Extremely low values have been observed in acute yellow atrophy or acute diffuse necrosis of the liver (15–50 mg.), acute and chronic infectious hepatitis and cholangiolitis, and in fatty liver. Marked hypoglycemia (25 mg.) has been reported in association with primary liver cell carcinoma replacing 70 to 80 per cent of the liver substance; at the time of death the blood sugar had fallen to 13 mg. per 100 cc. It is important to realize that other disturbances of function may not be demonstrable in spite of the fact that hepatic damage may be so extensive that the glycogen content of the liver is extremely low. The consideration of the state of the glycogen reserve is of the utmost importance in surgical disorders of the biliary tract since the administration of ether in such cases, by depleting this reserve, may result in serious consequences (postanesthetic hypoglycemia). Excessive, continued muscular exertion may be associated with hypoglycemia following the primary period of hyperglycemia, due likewise to secondary exhaustion of hepatic glycogen; this is also true of convulsive disorders such as occur in strychnine poisoning, eclampsia, uremia and tetanus.

Fasting hypoglycemia, usually mild but occasionally below 30 mg. per 100 cc., may occur in the hepatic type of glycogen storage disease (von Gierke's disease), usually in association with ketonuria and an increase in blood glycogen (over 15 mg. per 100 cc.) and plasma lipids. This condition is characterized by an excessive accumulation of glycogen in the liver and other organs due to, or at least associated with, an inability to mobilize this substance, as evidenced by a diminished blood sugar response to epinephrine (p. 54) and increased sensitivity to insulin (p. 53). The tendency toward fasting hypoglycemia is believed due to the abnormal stability of the hepatic glycogen stores, and develops rapidly when food is withheld. In some cases this increased stability may be due to the formation of liver glycogen of an abnormal type, structurally. In others, a deficiency in glucose-6-phosphatase has been demonstrated, an enzyme necessary for the final step in the conversion of glycogen to glucose in the liver. There thus appear to be different varieties of the hepatic type of glycogen storage disease. There is also a cardiac type, in which there is no significant abnormality of blood sugar or glucose tolerance, no ketosis, and a normal glycemic response to epinephrine.

Reference has been made (p. 34) to the occasional occurrence of hypoglycemia after administration of insulin to patients with hemochromatosis. The situation may be analogous to that of the hepatectomized-depancreatized dog, in which the blood sugar rises higher after glucose than in the depancreatized dog and hypoglycemic manifestations develop more rapidly than in hepatectomized animals. This phenomenon does not always occur, certain patients with hemochromatosis exhibiting an extreme degree of insulin resistance.

Adrenal Cortical Insufficiency. In Addison's disease, usually associated with atrophy or tuberculosis of the adrenal glands, the blood sugar is frequently low normal and occasionally subnormal. The average value in a large series of collected cases was 75 mg. per 100 cc. In a few instances figures of 30 to 40 mg. have been reported, usually in fatal cases shortly before death. This hypoglycemic tendency is due to diminished gluconeogenesis, increased utilization of glucose, and perhaps also to diminished absorption of glucose from the intestine, resulting from adrenal cortical insufficiency. The defect in carbohydrate metabolism may be demonstrated most readily by performing an intravenous glucose tolerance test, the characteristic finding being exaggeration and prolongation of the hypoglycemic phase of the blood sugar curve (p. 50). Insulin sensitivity is increased (p. 53).

Hypoglycemia (0–30 mg.), occurring as a terminal event in extensive burns, diphtheria and scarlet fever (adrenal apoplexy or Waterhouse-Friderichsen syndrome), has been attributed to suprarenal failure.

Anterior Pituitary Insufficiency. The disturbance in carbohydrate metabolism is similar to that in adrenal cortical insufficiency. It is due in large measure to the same mechanism, being accompanied by atrophy of the adrenal cortex resulting from absence of the adrenocorticotrophic hormone. In addition, there is atrophy also of the thyroid (absence of thyrotrophic hormone), which further increases the tendency toward hypoglycemia and increases the glucose tolerance and the insulin sensitivity (pp. 51, 53). Spontaneous hypoglycemia may occur, with convulsions and coma. Fasting blood sugar values of 60 mg. per 100 cc. or lower have been reported in about 50 per cent of verified cases of Simmonds' disease (pituitary cachexia) (range, 24–114 mg. per 100 cc.). A similar finding has been obtained in about 20 per cent of cases of anorexia nervosa (range, 26–134 mg. per 100 cc.), rendering the occurrence of spontaneous hypoglycemia of limited value in the differential diagnosis of these conditions.

A blood sugar of practically zero has been observed in a patient with diabetes mellitus following infarction of the hypophysis with destruction of the anterior lobe. Hypoglycemia has resulted from extensive destruction of the anterior hypophysis by a chromophobe adenoma.

Hypothyroidism. In myxedema and cretinism the blood sugar concentration may be low, values of 50 to 60 mg. per 100 cc. having been reported. Values of 60 to 70 mg. are commonly observed. A drop in blood sugar (50–60 mg. per 100 cc.) may occur at times immediately following thyroidectomy in individuals who have not received adequate amounts of carbohydrate before operation.

Nervous System Disorders. Fasting hypoglycemia, usually of mild degree, has been observed occasionally in vagotonia, "neurocirculatory asthenia," various psychoses, paresis, subarachnoid hemorrhage, the postencephalitic syndrome and certain brain stem (hypothalamic) lesions. Increased glucose tolerance (p. 52) occurs much more commonly than actual hypoglycemia in such cases. The mechanism underlying the abnormality in carbohydrate metabolism is believed to be disturbed nervous control of hepatic glycogenolysis, a similar condition having been produced experimentally by injury to the paraventricular nucleus in rabbits and the hypothalamic region in cats and dogs.

Miscellaneous. Mild hypoglycemia has been reported in cases of status thymicolymphaticus, progressive muscular atrophy, pregnancy, lactation, renal glycosuria, and in anorexia nervosa and other conditions accompanied by seever grades of undernutrition.

ABNORMAL ALIMENTARY RESPONSE

Exaggerated Response—Diminished Glucose Tolerance

By diminished glucose tolerance is meant inability of the organism to handle ingested glucose as efficiently as a normal organism; it indicates inefficiency of one or more of several factors involved in the metabolism of carbohydrate:

(1) Inadequate glycogen storage capacity of the liver, so that glucose reaching the liver in the portal blood is not adequately removed, and, as a result, enters the systemic circulation in abnormal amounts. The chief clinical conditions in which this factor plays an important part are those in which there is extensive and rapidly progressive hepatic disease.

(2) Increased hepatic glycogenolysis and gluconeogenesis. In this group are included hyperthyroidism, hyperadrenalinism, acidosis and toxemia due to acute

infections, and adrenal cortical hyperfunction. These phenomena probably contribute also to the decreased glucose tolerance of diabetes mellitus. In the past, the generally accepted view has been that infection or toxemia affects the pancreas in such a way as to diminish insulin production or that they interfere with the action of insulin, whether of endogenous or exogenous origin. More recent studies have led to the hypothesis that the diminished glucose tolerance and relatively high insulin resistance associated with toxemia and infection are dependent, in part at least, upon acceleration of hepatic glycogenolysis. Some believe this to be due to increased epinephrine secretion, but it may be due to interference with the mechanism whereby the liver normally diminishes its supply of glucose to the blood in response to an influx of exogenous sugar (p. 22). A similar mechanism may also operate in the presence of hepatic functional impairment associated with various types of biliary tract disorder and liver disease included in the preceding group.

(3) Decreased tissue utilization. If the ability of the extrahepatic tissues to form glycogen and to utilize glucose is diminished, an excess remains in the blood. The most important clinical condition in this group is diabetes mellitus (hypoinsulinism).

(4) Increased rate of absorption. An abnormally high rise in the blood sugar level may result from marked increase in the rate of absorption of glucose from the intestine, believed by some to occur in hyperthyroidism.

The chief features of the blood sugar curve following the ingestion of glucose (Fig. 9) which characterize a diminished glucose tolerance are:

(*a*) An abnormally high rise in the venous blood sugar concentration (above 160 mg. per 100 cc.).

(*b*) The maximum concentration is reached later than in the normal individual (after one hour).

(*c*) The period of hyperglycemia is longer than normal.

(*d*) The return to the postabsorptive level is delayed (more than two hours).

In conditions in which the dominant factors are abnormally rapid absorption (e.g., hyperthyroidism), inadequate storage in the liver (e.g., hepatic disease) or excessive hepatic glycogenolysis (e.g., hyperadrenalinism, acidosis, toxemia), the most marked abnormality occurs in the first phase of the glucose tolerance curve; i.e., there is an excessively high rise in the blood sugar, but the return to the control level is not as long delayed as in conditions in which there is believed to be impaired tissue storage and utilization of glucose. The latter condition is observed characteristically in diabetes mellitus, the blood sugar curve being abnormally high and markedly prolonged.

Diabetes Mellitus (p. 67). Diminished glucose tolerance is the most characteristic metabolic feature of this condition, occurring regardless of the postabsorptive blood sugar concentration. It is probably dependent in part upon disturbance of the homeostatic mechanism in the liver (p. 22), resulting in continuation of hepatic glycogenolysis and gluconeogenesis at abnormally high blood sugar levels, and also upon diminished capacity of the tissues for storing and utilizing glucose at normal blood sugar levels.

The sugar tolerance curve in diabetes mellitus has the following characteristics:

(*a*) Fasting hyperglycemia is present in all but very mild cases.

(*b*) A gradual rise to an excessively high level (above 160 mg. per 100 cc.), the degree of elevation being approximately proportional to the severity of the condition.

(*c*) The maximum concentration is reached after a variable interval following the ingestion of glucose, but practically always more than one hour. In general, the greater the rise, the longer is the time elapsing before the highest level is attained. In severe cases the peak may not be reached for three or more hours.

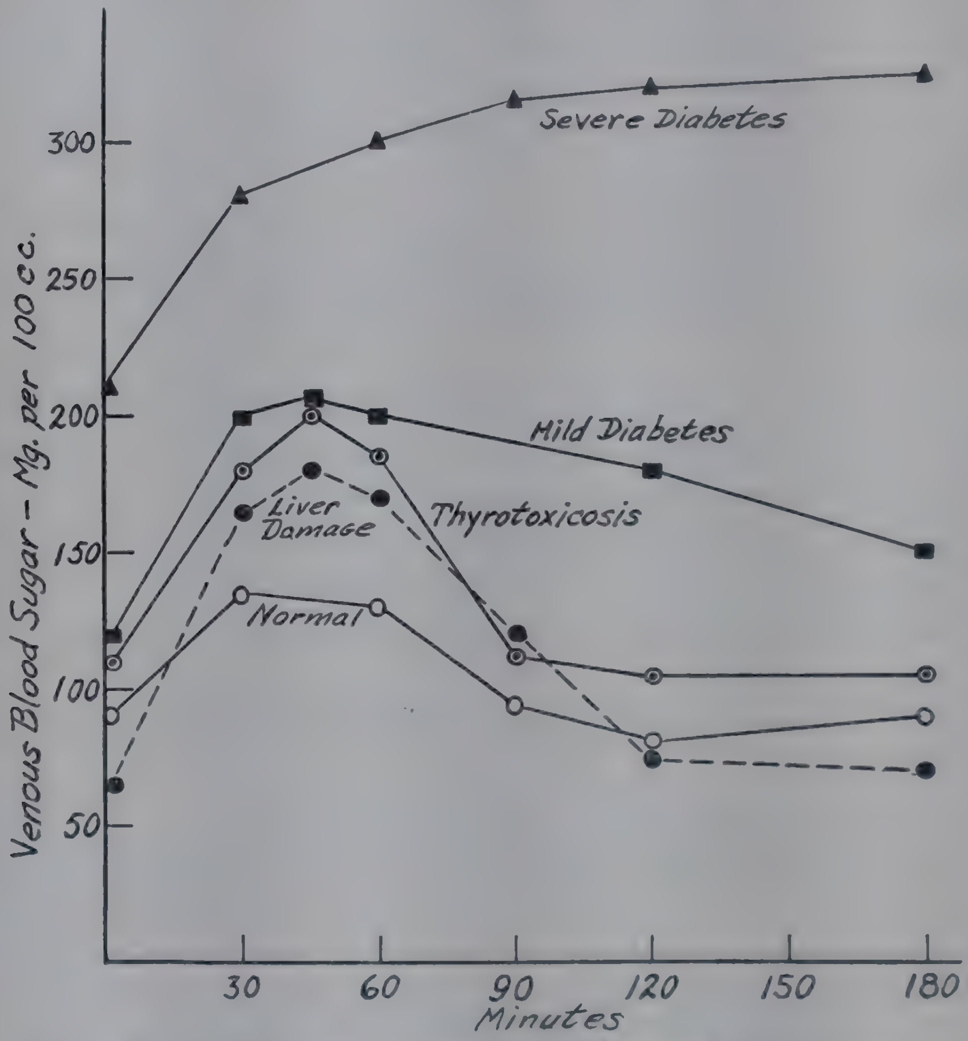


Fig. 9. Blood sugar curves characteristic of decreased glucose tolerance (100 gm. glucose by mouth).

(*d*)The most characteristic feature is the delayed return to the postabsorptive level. A failure to return to normal at the end of three hours is usually indicative of diabetes mellitus. After reaching a maximum, the blood sugar concentration remains at a high level for a variable period, decreasing slowly to the fasting level. The greater the elevation the slower is the rate of decrease, both paralleling the severity of the condition. Characteristically the curve is of the high plateau rather than the peaked type.

(*e*) The arterial-venous difference is often diminished.

Other phenomena may occur which suggest impaired glucose utilization. The respiratory quotient may remain at the postabsorptive level (p. 32) instead of exhibiting the normal increase which attends active carbohydrate storage and combustion. However, this may also be interpreted as due to increased gluconeogenesis from protein and fat. The serum phosphate concentration characteristically remains unchanged instead of decreasing during the period of hyperglycemia; in some cases it may increase slightly.

The glucose tolerance test is of particular diagnostic value in cases in which the fasting blood sugar is normal or only slightly elevated. The intravenous test is practically never indicated in cases in which diabetes mellitus is suspected and, since interest is centered chiefly in the first phase of the curve, the test may usually be terminated at the end of two or three hours for all practical purposes in such cases.

ABNORMAL ONE-HOUR, TWO-DOSE TEST. Exton found that in the one-hour, two-dose test in mild diabetes, the first part of the curve is similar in trend to that obtained in the ordinary one-dose glucose tolerance test. After the second dose of glucose, however, the blood sugar rises sharply, this feature distinguishing diabetes from all other conditions, according to this author. In more severe diabetes the responses to the one-hour, two-dose test are in general the same as the responses in mild diabetes, except that the sugar values are higher in all the blood and urine samples in the more severe cases. According to Exton, the criteria for determining diabetes in the one-hour, two-dose test are (1) a more or less steep rise of not less than 10 mg. of blood sugar in the sixty-minute sample, following the second dose of glucose, and (2) the relation of blood and urine sugar values to the severity of the disease. The criteria of alimentary glycosuria are (1) a sugar-free urine after fasting, with sugar in the final urine specimen, and (2) blood sugars that follow the normal curve even when the level is higher than normal. The criteria for renal glycosuria are (1) blood sugars which follow the normal course, or in any event which never reach the diabetic level, and (2) sugar in both urine specimens.

According to some, this procedure is more valuable than the ordinary glucose tolerance test in that it frequently yields normal findings in nondiabetic subjects in whom abnormal or questionable results are obtained by the latter method. In our experience with hospital patients, suffering from a variety of disorders, this procedure has not proven superior to the older test. Distinctly abnormal curves were obtained in many patients in whom no evidence of diabetes could be demonstrated and in whom essentially normal curves were obtained by the single dose procedure. In our experience, the most reliable criterion for the diagnosis of diabetes with this procedure is a one-hour blood sugar above 160 mg. per 100 cc.

Hepatic and Biliary Tract Disease. If the sugar which enters the blood after the ingestion of glucose is removed both by the tissues and by the liver, one should theoretically expect definite abnormalities in the curve of alimentary glycemia in persons with liver disease. Because such individuals partially or totally lack one of the mechanisms that reduce hyperglycemia, hepatic glycogen formation, the curve would presumably be excessively high or prolonged. Unfortunately, however, from a diagnostic standpoint, this theoretical observation is not always borne out in fact. A normal alimentary response may be obtained in the presence of advanced hepatic disease. This is particularly true of chronic disorders such as various types of cirrhosis, passive congestion, carcinoma, and lues of the liver. This is in all probability due to the great functional reserve and remarkable regenerative power of that organ. In the acute diffuse forms of hepatic disease mentioned below, significant alterations in the blood sugar tolerance curve occur much more frequently. Such abnormalities have been described in acute and subacute hepatic necrosis, various forms of hepatitis, fatty liver, cholangiolitis, obstructive jaundice, acute alcoholism, portal cirrhosis and poisoning with phosphorus, chloroform, carbon tetrachloride, sulfonamides and arsphenamines. An abnormally high rise, occasionally prolonged, with an exaggerated hypoglycemic phase, has been observed in glycogen storage disease (von Gierke's disease) (p. 57).

The type of curve obtained in typical cases of insufficiency of hepatic glycogenic function is characterized by these factors:

(*a*) Abnormally high rise in venous blood sugar concentration (above 160 mg.).

(*b*) The maximum concentration is attained at the end of three-fourths to one and one-half hours, and in most instances within one hour.

(*c*) The blood sugar level usually falls rather rapidly, returning to normal within two to three hours. Only in cases of extreme grades of hepatic insufficiency is hyperglycemia of long duration.

(*d*) The arterial-venous difference is usually normal or, in some instances, increased.

(*e*) The combination of a low normal or subnormal fasting blood sugar level with a tolerance curve having the characteristics described above is highly suggestive of diminished hepatic glycogenesis.

If determinations of the respiratory quotient and serum phosphate are made at frequent intervals after the administration of glucose, they will be found to change in an essentially normal manner; i.e. the R.Q. rises and the serum phosphate falls during the period of increased glucose supply and utilization (see p. 32).

Hyperthyroidism. Decreased tolerance for glucose occurs in 50 to 80 per cent of patients with hyperthyroidism, but does not necessarily parallel the severity of the condition. It is probably due in part to an increased rate of absorption from the intestine and in part to increased hepatic glycogenolysis. The blood sugar curve is not as characteristically plateau in type as that in diabetes mellitus, usually rising to a high peak earlier and descending earlier than in the latter condition.

Hyperfunction of the Anterior Pituitary and Adrenal Cortex. Cushing's syndrome, whether due to primary hyperfunction of the anterior pituitary (basophilic adenoma or hyperplasia) or primary adrenal cortical tumor or hyperplasia, is characteristically accompanied by a state of decreased glucose tolerance. This is not present in all cases in early stages of the condition, but develops eventually almost invariably. It is dependent largely upon excessive gluconeogenesis and decreased glucose utilization (pp. 16, 70) and, in the case of pituitary hyperfunction, perhaps also upon effects exerted through the thyroid. The curve in mild cases may resemble that seen in hyperthyroidism, acute infections, "toxemia," etc., but in more severe cases it is usually of the characteristic diabetic type. This so-called "steroid diabetes" is characterized by relative insensitivity to insulin, mildness during fasting, and negative nitrogen balance in the absence of marked glycosuria.

The glucose tolerance varies considerably in acromegaly, but is usually decreased at some phase of the development of the condition. In advanced cases, with destruction of the remainder of the gland by the eosinophilic tumor, normal curves may be obtained. The variable findings may also be due to opposing influences in this connection of factors stimulating gluconeogenesis from protein and of the factor or factors stimulating growth and thus tending to conserve protein.

Glucose tolerance is usually normal in the adrenogenital syndrome, characterized by precocious puberty with either isosexuality in children, masculinization in the female or feminization in the adult male. In some instances, however, a diabetic type of curve may be obtained. Patients with adrenal medullary hyperfunction (pheochromocytoma) exhibit decreased tolerance for glucose during, and at times between, the characteristic attacks of paroxysmal hypertension and hyperglycemia (p. 35).

Pregnancy. Mild to moderate decrease in glucose tolerance, with a normal fasting blood sugar, occurs rather commonly in the last trimester in otherwise apparently normal women. The question naturally arises as to whether this is due entirely to the physiological alteration in endocrine function or whether it is a reflection of a diabetic tendency which was dormant in the nonpregnant state. This distinction is of great importance because of the very high infant mortality in diabetes.

In general, the earlier the appearance of decreased glucose tolerance (e.g., in the first or second trimesters), the more severe the abnormality, particularly if there is an associated elevation of the fasting blood sugar concentration, and the more justified one is in regarding the condition as diabetes mellitus and in instituting treatment. There is experimental basis for the belief that early correction of the periodic hyperglycemia by insulin may prevent the development of frank diabetes mellitus in the mother and reduce the fetal mortality.

Miscellaneous. Diminished tolerance to glucose is commonly observed in patients with severe anemia, including pernicious anemia. It appears to occur most consistently in cases in which the free HCl of the gastric juice is low or absent. Some observers have reported an improvement in glucose tolerance associated with or following administration of HCl. Diminished tolerance occurs commonly also in patients with essential hypertension.

Fasting hyperglycemia and diminished glucose tolerance are at times observed in patients with glomerulonephritis and nephrosclerosis, particularly in the presence of renal failure. There is no associated disturbance of glucose utilization, the respiratory quotient rising in a normal manner following the ingestion of glucose. The abnormality apparently lies in a retarded transfer of glucose from blood to tissues rather than in retarded combustion of glucose in the tissues.

Decreased glucose tolerance may occur in certain central nervous system disorders, including head injuries or other causes of increased intracranial pressure (brain tumor, abscess, hemorrhage), hypothalamic lesions, emotional instability and, at times, dementia praecox and manic-depressive insanity.

It also occurs in about 75 per cent of cases of hemochromatosis (fibrosis and degeneration of the islands of Langerhans) and occasionally in acute and chronic pancreatitis, due to involvement of the islands of Langerhans and to associated hepatic and biliary tract disease.

Various infectious and toxic states often depress glucose tolerance. This is particularly true of pyogenic infections, especially of the skin (furunculosis, carbuncle), but diminished tolerance may also occur in such conditions as rheumatoid arthritis, diphtheria, scarlet fever, pneumonia, tuberculosis, and others. The influence of infections is perhaps exerted through the medium of the liver, i.e., by decreased responsiveness of the mechanism of hepatic glycogenolysis and gluconeogenesis to an increase in the blood sugar concentration (p. 22).

Curves indicative of diminished tolerance may be obtained in subjects with simple obesity (improving after weight-reduction), essential hypertension, arteriosclerosis, and Paget's disease (osteitis deformans), in about 50 per cent of cases of advanced malignancy and certain other conditions accompanied by severe undernutrition, in infants with extreme dehydration and in subjects in whom the carbohydrate intake has been restricted prior to the performance of the test.

Decreased Response—Increased Glucose Tolerance

By increased glucose tolerance is meant increased ability of the organism to handle glucose. The alimentary blood sugar response may be affected in one or both of two ways: (*a*) the initial blood sugar rise (hyperglycemic phase) is abnormally low or, at times, entirely absent, and/or (*b*) the hypoglycemic phase is abnormally pronounced and prolonged. In the majority of cases the latter phenomenon is elicited most readily by means of the intravenous glucose tolerance test. Spontaneous hypoglycemia may or may not be present. Subjects with increased tolerance for glucose almost invariably also exhibit increased sensitivity to insulin.

Hyperinsulinism. As indicated elsewhere (p. 39), this condition may occur as a result of simple hyperfunction, hyperplasia, adenoma or carcinoma of the islands of Langerhans of the pancreas. The metabolic phenomena may be considered the result of a constant and excessive supply of insulin instead of a periodic secretion in response to the requirements of the organism. The characteristic blood sugar curve following administration of glucose should theoretically resemble that which follows the simultaneous administration of glucose and a large dose or repeated doses of insulin.

(*a*) Fasting hypoglycemia.

(*b*) Little or no elevation of blood sugar following glucose ingestion, resulting in a flat type of curve.

(*c*) Exaggeration of the hypoglycemic phase.
(*d*) Decrease in serum phosphate.
(*e*) Increase in the respiratory quotient.

However, the glucose tolerance curves in many cases of hyperinsulinism do not conform exactly to this description. In some cases, the blood sugar concentration may rise to excessive heights (over 250 mg.). This observation, suggestive of diminished sugar tolerance, has been interpreted as due to saturation of the liver with glycogen. Numerous studies in such cases have shown that the glucose tolerance test of three or four hours, as ordinarily carried out, is usually of little value in establishing a diagnosis of hyperinsulinism. Although the low blood sugar values in the fasting state are significant and should arouse suspicion, the normal or even excessive rise during the first hour and the subsequent fall to a relatively normal level within three hours may be misleading. However, if one continues to make observations of the blood sugar concentration over a period of six hours, much more characteristic findings are usually obtained. After reaching a normal level, the blood sugar continues to fall and may be extremely low at the end of five to six hours. This procedure should be carried out in all cases of suspected hyperinsulinism.

Abnormal prolongation of the hypoglycemic phase of the curve is of much greater significance than is the degree of hypoglycemia. This phenomenon is usually elicited best by the intravenous glucose tolerance test. The shape of the curve is influenced considerably by the nature of the antecedent diet, a high carbohydrate intake for a few days before the test favoring the production of a flat type of curve and a previously low carbohydrate intake often causing a normal or supernormal initial hyperglycemic phase. The insulin tolerance test (p. 53) is almost invariably abnormal, indicating increased sensitivity to insulin. In some instances, removal of an adenoma of the pancreatic islet cells is followed by a temporary state of hypoinsulinism, with diminished glucose tolerance, due probably to suppressed function of the islets of Langerhans.

In certain cases exhibiting manifestations similar to those indicated above no morphologic abnormality of the pancreatic islet cells can be demonstrated. These have been designated "functional hyperinsulinism," but the justification for this appellation is questionable. In this condition, the hypoglycemic attacks, although occurring usually two to four hours after meals, appear to be related more directly to emotional states or physical stress, are more transient and less inclined to progress in severity and frequency than in organic hyperinsulinism. The hypoglycemic phase of the glucose tolerance curve is less prolonged, as is the hypoglycemia induced by insulin or by insulin plus glucose. Autonomic imbalance in the direction of vagotonia may play a part in the pathogenesis of this type of hypoglycemia.

Adrenal Cortical Insufficiency. This condition is characteristically associated with (*a*) increased tolerance for glucose, (*b*) increased sensitivity to insulin and (*c*) a decreased glycemic response to epinephrine. Some or all of the following abnormalities of carbohydrate metabolism have been observed in large proportion of cases of Addison's disease.

(1) Low-normal or subnormal fasting blood sugar concentration (p. 41).

(2) Flat type of oral glucose tolerance curve, i.e., diminished glycemic response after ingestion of glucose. This is due, in part at least, to a diminished rate of absorption of glucose from the intestine, which is the only abnormality of carbohydrate metabolism that is corrected by administration of desoxycorticosterone acetate. Intravenous administration of glucose under standardized conditions (p. 25) is usually followed by a rise in blood sugar to an essentially normal level, i.e., the hyperglycemic phase of the curve is essentially normal.

(3) Exaggeration of the hypoglycemic phase of the curve after either oral or, more strikingly, intravenous administration of glucose. The blood sugar falls to an abnormally low level at two to three hours and may remain at this level for several hours instead of returning promptly to the control level, as in normal subjects. During this period, severe subjective and objective manifestations of hypoglycemia often develop at a higher blood sugar concentration than in the case of normal subjects.

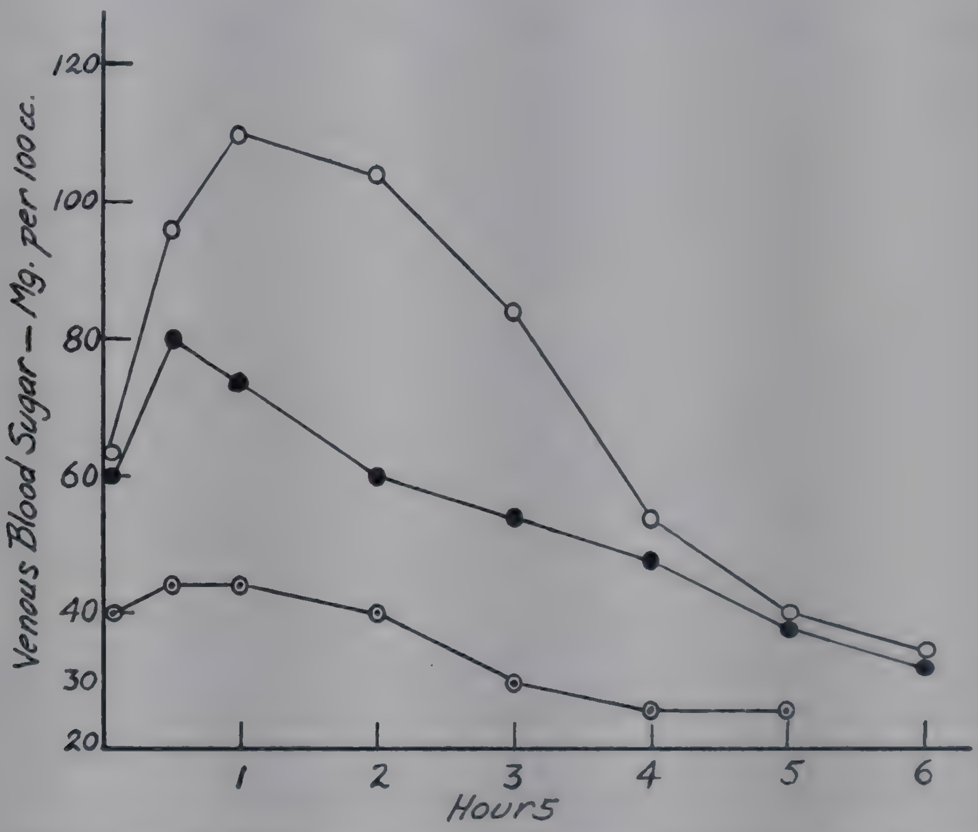


Fig. 10. Three types of blood sugar response to ingestion of 100 gm. of glucose in cases of hyperinsulinism.

(4) Marked prolongation of the hypoglycemic response to insulin (p. 53), i.e., "hypoglycemia unresponsiveness."

(5) Slightly decreased glycemic response to a standard dose of epinephrine.

(6) Striking hypoglycemia (*a*) during fever or infections, (*b*) on a diet high in fat and low in carbohydrate, and (*c*) following a twenty-four-hour fast.

(7) High standard R.Q. (p. 32), which increases more than normally after administration of glucose.

These defects in carbohydrate metabolism are due to (*a*) diminished absorption of glucose from the intestine and (*b*) decreased gluconeogenesis from protein (amino acids) (p. 9). The ability to form glucose from lactic acid and pyruvate as well as from glucogenic amino acids is apparently impaired. The diminished absorption can be corrected by administration of an adequate amount of sodium chloride or desoxycorticosterone acetate. This, as well as the more fundamental defects in gluconeogenesis, glucose utilization, and glycogenesis, can be corrected by adequate amounts of adrenal cortical extract or 11-oxygenated adrenal hormones (e.g., Compounds E and F) (p. 424).

The adrenal cortical hormones which exert an effect upon carbohydrate metabolism are those which contain an oxygen atom attached to the 11-carbon atom. Desoxycorticosterone, which does not possess this feature, is without significant influence upon carbohydrate metabolism, although it exerts a striking effect upon salt and water metabolism (pp. 285, 432).

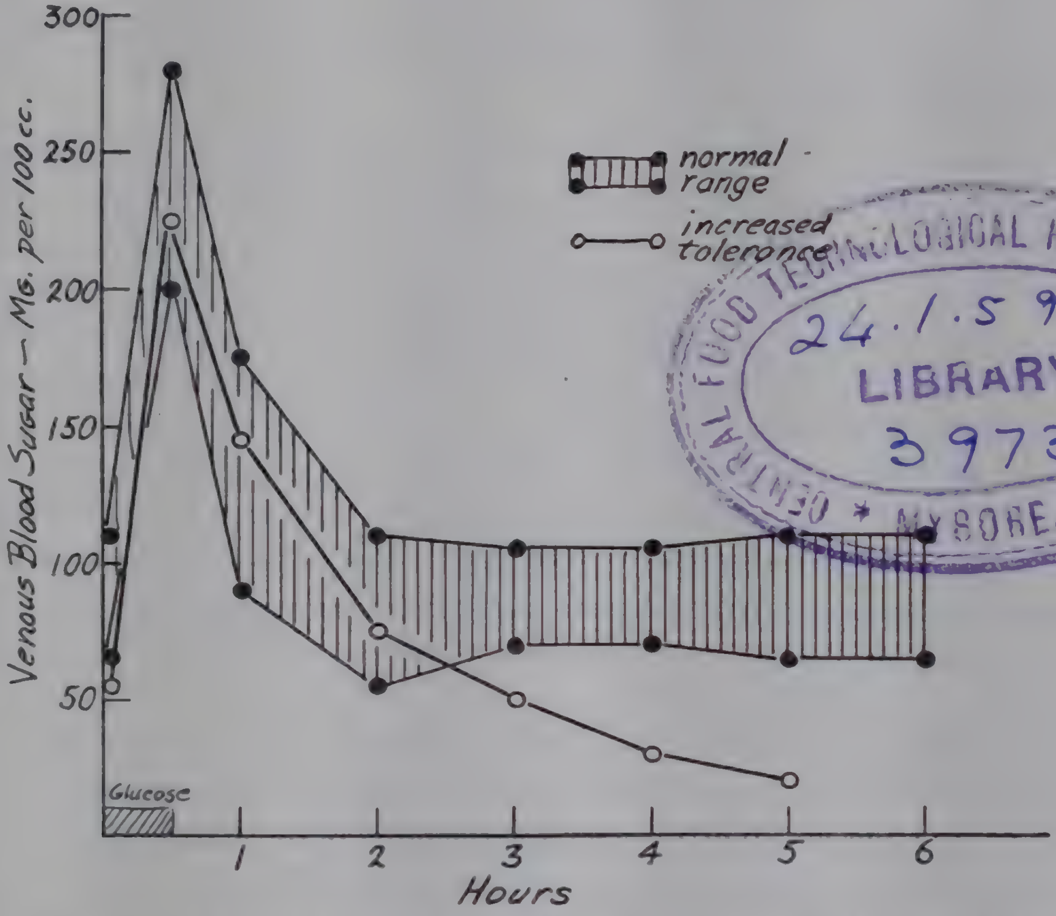


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Fig. 11. Intravenous glucose tolerance curves. Curve characteristic of increased tolerance is obtained in typical cases of Addison's disease and hyperinsulinism.

Because of the ease with which hypoglycemic manifestations are produced in subjects with adrenal cortical insufficiency, provision must be made for prompt intravenous administration of glucose when either glucose or insulin tolerance tests are performed.

Anterior Pituitary Hypofunction. Increased tolerance for glucose (flat blood sugar curve) was reported in 52 per cent of twenty-one verified cases of pituitary cachexia (Simmonds' disease) due to destructive lesions, such as atrophy, hemorrhage, chromophobe tumor, and cyst, normal curves being obtained in 38 per cent and diabetic curves in 10 per cent. It is difficult in such cases to evaluate

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the influence of severe undernutrition in this connection, as evidenced by the occurrence of a flat type of curve in about 30 per cent of cases of anorexia nervosa. However, in Simmonds' disease, there is characteristically a tendency toward spontaneous hypoglycemia, with increased glucose tolerance, similar to that in Addison's disease, and increased sensitivity to insulin.

The glucose tolerance is not so frequently altered in other types of pituitary hypofunction, such as pituitary infantilism or dwarfism. Although the glucose tolerance may be increased, fasting hyperglycemia and decreased glucose tolerance have been observed in such cases.

Hypothyroidism. A flat type of glucose tolerance curve is commonly obtained in cretinism and myxedema. This is due in part to a diminished rate of absorption from the intestine, for the intravenous glucose tolerance test yields essentially normal findings in many such cases.

It is interesting in this connection that the fasting blood sugar concentration has been found to be normal in nondiabetic patients with hypothyroidism induced by total thyroidectomy. However, the post-operative sugar values tend to be slightly lower than those obtained before operation. Similarly, although the hyperglycemia produced by glucose ingestion is usually slightly less after total thyroidectomy than previously, the glucose tolerance curve is generally within normal limits in nondiabetic patients with induced hypothyroidism. Carbohydrate metabolism is apparently not significantly influenced by hypothyroidism induced by total thyroidectomy except when a derangement of carbohydrate metabolism is evidenced prior to operation. Marked improvement in glucose tolerance has been observed in diabetic patients following removal of the thyroid gland, and also in patients with hyperthyroidism who present a diabetic type of curve before operation.

Miscellaneous. A flat type of glucose tolerance curve may be obtained in renal glycosuria, in hypothalamic lesions, in some, but not all cases of glycogen disease (p. 57), due apparently to increased stability of the hepatic glycogen, with diminished glycogenolysis, and in idiopathic steatorrhea (celiac disease, sprue), probably as a result of impaired absorption of glucose from the intestine. Curves of this nature may be produced by conditions associated with increased intestinal motility, such as vitamin B deficiency, "gastrointestinal neuroses," achlorhydria, tuberculous enteritis, and chronic ulcerative or mucous colitis. In such cases, a normal response is obtained with the intravenous glucose tolerance test. Curves indicative of increased tolerance have been reported in marasmus in infants and in anorexia nervosa, and may be produced in normal subjects by excessive carbohydrate feeding prior to the performance of the test. Hypoglycemia, occurring about two hours after meals, has been observed in partially gastrectomized subjects. Oral glucose tolerance tests in such individuals reveal an excessively high rise and exaggeration of the subsequent hypoglycemic phase of the curve. This has been attributed to the rapid passage of the glucose into the intestine (dumping syndrome), its rapid absorption, and excessive stimulation of insulin secretion due to the abnormally high rise in blood sugar.

Abnormal Insulin Tolerance

The purpose of the insulin tolerance test is to determine (*a*) the sensitivity of the organism to insulin and (*b*) its responsiveness to insulin-induced hypoglycemia. The normal blood sugar response to insulin is discussed elsewhere (p. 31). Abnormalities may occur in one or both of two phases of the curve, i.e., in the extent and duration of the hypoglycemia and in the subsequent rise toward the pre-injection level. There are two important types of abnormality. (1) "Insulin resistance," characterized by a relatively slight (less than 50 per cent of the control level) or delayed (45 minutes or longer) fall in blood sugar; (2) "Hypoglycemia unresponsiveness," characterized by undue prolongation of the period of hypoglycemia (absence of or marked delay in the subsequent rise in blood sugar).

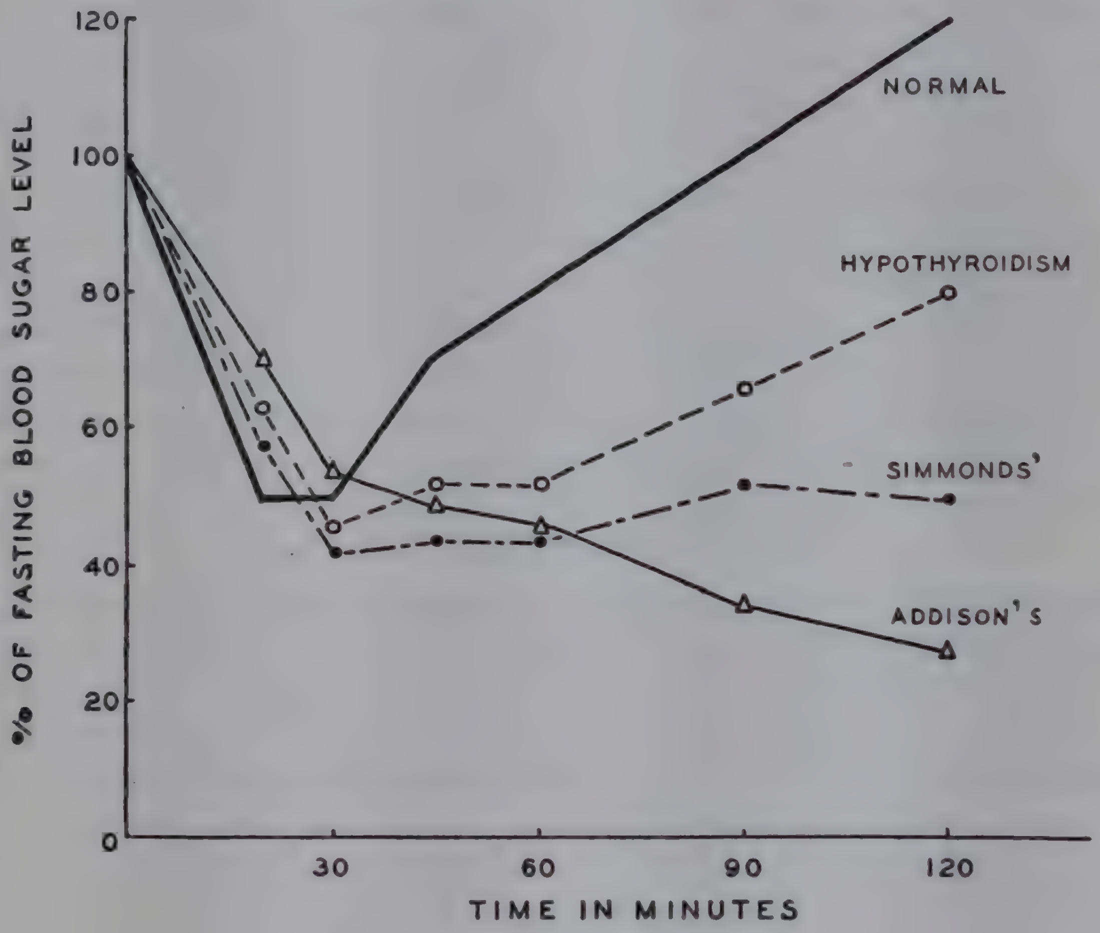

Fig. 12. Insulin tolerance test.

Hypoglycemia unresponsiveness is observed characteristically in hyperinsulinism, Addison's disease (adrenal cortical hypofunction), anterior pituitary hypofunction (Simmonds' disease and, at times, pituitary infantilism or dwarfism), and in some cases of glycogen disease. When performing this test in suspected cases of these disorders, provision must be made for prompt intravenous administration of glucose if serious manifestations of hypoglycemia develop, or to terminate the prolonged period of hypoglycemia. This procedure may be useful in differentiating anterior pituitary hypofunction, as in Simmonds' disease, from anorexia nervosa and, at times, from primary hypothyroidism.

Insulin resistance may be observed in primary adrenal cortical hyperfunction (Cushing's syndrome), anterior pituitary hyperfunction (Cushing's syndrome, acromegaly) and in some cases of diabetes mellitus. Subjects with diabetes mellitus have been classified as "insulin-resistant" and "insulin-sensitive" partly on the basis of this test and partly on the basis of the glucose-insulin tolerance test. Excluding "steroid diabetes" (adrenocortical or pituitary hyperfunction), the validity of this classification is questionable. There are, of course, instances of true insulin resistance, but this phenomenon is not related to the pathogenesis of the diabetic state in such cases.

Hypoglycemia unresponsiveness can usually be demonstrated by means of the combined insulin-glucose tolerance test (p. 31), with less danger of undesirable reactions than with the conventional insulin tolerance test. In normal subjects, hypoglycemia induces a homeostatic response which results in prompt rise in blood sugar to a normal level (i.e., decrease in insulin secretion, increase in hepatic glycogenolysis). If glucose is administered in the hypoglycemic state, the subsequent rise in blood sugar, in normal subjects, exceeds that which occurs if glucose is given at normal blood sugar levels. In hypoglycemia unresponsiveness, this phenomenon is not observed, characteristically, the response to glucose being usually less than that seen normally in the conventional glucose tolerance test. Equivocal results have been reported with this procedure in occasional cases of anorexia nervosa and psychogenic vomiting.

Abnormal Epinephrine Tolerance Test

Diminished glycemic response to epinephrine (rise of less than 35 mg. per 100 cc. in 40 to 60 minutes) may occur in conditions in which the hepatic glycogen stores are depleted (in the absence of other disturbances of carbohydrate metabolism), particularly in advanced grades of hepatocellular damage (acute and subacute hepatic necrosis, various forms of hepatitis, fatty liver, cirrhosis, and so on). It is also observed characteristically in patients with glycogen disease (von Gierke), in which condition the liver contains an excessive quantity of glycogen which appears to be highly resistant to glycogenolytic stimuli. A subnormal response to epinephrine also occurs commonly in conditions in which a state of "hypoglycemia unresponsiveness" is demonstrated by means of the insulin tolerance test, viz., hyperinsulinism, Addison's disease, and pituitary cachexia.

Abnormal Tolerance for Other Sugars

Abnormal Fructose Tolerance. The metabolism of fructose differs in several essential details from that of glucose (p. 29). Although fructose may be utilized without preliminary transformation to glucose or glycogen, it is probably largely removed from the blood and transformed into glycogen before contributing to the blood glucose or possibly before undergoing oxidation. The liver appears to be the chief site of transformation of fructose to glycogen. Indeed, it appears that fructose is a better glycogen-former than glucose and that it can undergo this transformation in the absence of insulin. Because of the specific importance of the liver in the intermediary metabolism of this sugar, the determination of the tolerance of the organism for fructose has been suggested as a means of detecting impairment of hepatocellular function (p. 500). Following the ingestion of 45 gm. of fructose, (p. 30), diminished capacity of the liver for transforming this sugar to glycogen or glucose is evidenced by the following phenomena:

(*a*) A rise in blood sugar of more than 35 mg. per cent, a concentration of 135 mg. or more being reached at some period during the performance of the test.

(*b*) A delayed return to the postabsorptive level (beyond two hours).

The demonstration of diminished fructose tolerance in the absence of evidence pointing to any disturbance of general carbohydrate metabolism is suggestive of impairment of liver function. However, negative results are commonly obtained in the presence of advanced hepatic disease especially if chronic in nature (cirrhosis, lues and malignancy of the liver, etc.). Positive results may be obtained in more acute forms of liver disease, such as acute yellow atrophy, toxic necrosis or hepatitis associated with chloroform, arsenic, phosphorus and carbon tetrachloride poisoning, infectious hepatitis, etc. As a general rule this test is of little practical clinical value in the estimation of liver functional efficiency since in most instances other evidence of functional impairment is present long before positive results are obtained. Of still less value is the occurrence of fructosuria following the ingestion of 100 gm. of fructose. At least 10 per cent of normal individuals respond by eliminating some fructose in the urine and many patients with hepatic disease yield findings within the limits regarded as normal.

More satisfactory results have been reported when the analytical procedure is modified so that the blood fructose alone is determined, rather than fructose plus glucose. In the absence of hepatocellular damage, after ingestion of 50 gm. of fructose, the blood fructose concentration, usually 0 to 8 mg. per 100 cc. in the fasting state, increases not more than 15 mg. per 100 cc., usually within the first hour, and falls to 0 to 10 mg. at the end of two hours. Increases of 16 to 30 mg., with a delayed fall to the resting level, have been observed in patients with hepatocellular damage. Similar findings have been obtained at times in patients with arteriosclerosis.

Abnormal Galactose Tolerance. In view of the widespread employment of the galactose tolerance test as a measure of liver function, interest centers particularly upon the part played by the liver in the intermediary metabolism of this sugar. It has been found that when galactose is injected intravenously in the normal dog it disappears from the blood in about two hours, 10 to 30 per cent appearing in the urine. Removal of 50 to 70 per cent of the liver has little effect upon the tolerance for galactose. In the absence of the liver, 50 to 60 per cent of the amount injected is recovered in the urine. Similarly, increased excretion has been observed in the presence of acute degenerative lesions of the liver produced experimentally by carbon tetrachloride, chloroform, phosphorus, and similar hepatotoxic agents.

The usefulness of this procedure as a measure of hepatocellular function and as a means of differentiating between obstructive and hepatocellular jaundice is discussed in detail elsewhere (p. 501). Suffice it to state here that, with the oral test, the excretion by a jaundiced patient of less than 3 gm. of galactose in five hours suggests that the jaundice is not of hepatocellular origin, while the urinary excretion of more than 4 to 5 gm. suggests that it is. With the intravenous test, the presence of more than 20 mg. of galactose per 100 cc. of blood at the end of seventy-five minutes, in a patient with jaundice, suggests that the latter is of hepatocellular origin, while less than 20 mg. per 100 cc. at this time points toward obstructive jaundice.

Determination of the increase in blood galactose after ingestion of 40 gm. of this sugar has been proposed as a means of studying thyroid function. It has been found that in hyperthyroidism the peak of blood galactose concentration ranges from 25 to 150 mg. per 100 cc., being above 40 mg. in the great majority of cases (normal maximum 15–35 mg. per 100 cc.). This abnormally high rise is attributed to increased rapidity of absorption of the sugar from the intestine in patients with hyperthyroidism. Essentially normal results are obtained in hyperthyroidism when the galactose is given intravenously, indicating that the above findings are not due to abnormality of intermediary metabolism. This procedure may be of value in differentiating hyperthyroidism from other conditions accompanied by increase in the basal metabolic rate that may simulate it clinically, especially congestive heart failure of nonhyperthyroid origin.

Decreased tolerance for galactose has been observed in hyperpituitarism, certain pluriglandular disturbances involving gonadal dysfunction, and occasionally during menstruation and after the menopause.

BLOOD LACTIC ACID AND PYRUVIC ACID

Under resting conditions there are small amounts of lactate (5–20 mg./100 cc.) and pyruvate (0.4–2 mg./100 cc.) in the blood plasma. These intermediates in carbohydrate metabolism are derived mainly from glycolytic reactions (p. 5) in the muscles and erythrocytes. Lactate increases (up to 100 mg.), as does pyruvate, to a lesser degree (up to 5 mg.), in strenuous muscular exercise, when the requirement for energy outstrips the supply of oxygen to the muscles, in which the metabolic emphasis is therefore on anaerobic glycolysis, with excessive production of lactic acid; this passes into the blood stream, destined for reconversion to glycogen and glucose in the liver (p. 9).

Relatively slight exertion produces an abnormally high level of blood lactate and pyruvate in conditions associated with anoxia, e.g., congestive heart disease, valvular or hypertensive, and coronary artery disease. This is of little practical consequence, although it has been proposed as an objective means of evaluating circulatory efficiency or myocardial reserve.

Increase in blood lactate in patients in shock is of serious prognostic significance, indicating the onset of the irreversible stage of shock.

Thiamine pyrophosphate is an essential coenzyme (cocarboxylase) in the transformation of pyruvate to acetate (p. 8). In thiamine deficiency, therefore, pyruvate, and its reduction product, lactate, accumulate in excess and their concentrations in the blood increase. Determination of blood pyruvate has been employed for the detection of thiamine deficiency, but is obviously not specific for this purpose.

Although the liver is the organ chiefly concerned in the removal of lactate from the blood, the plasma lactate concentration is seldom increased in hepatic disease except, occasionally, in severe, acute hepatic necrosis or in terminal stages of decompensated cirrhosis. However, injected lactate may not be removed from the blood at normal rate in patients with liver damage; consequently, a lactate tolerance test has been proposed as a test of liver function. It has not been widely employed, and has no advantage over several simpler procedures.

In normal subjects, administration of glucose is followed by a significant increase in blood lactate and pyruvate within one hour, a manifestation of increased glucose utilization. This also follows injection of insulin. In diabetes mellitus, this rise is delayed or absent, and, therefore, determination of blood lactate and pyruvate in their relation to changes in blood sugar in the course of the glucose tolerance test has been proposed as a means of distinguishing between pancreatic diabetes and other conditions exhibiting decreased glucose tolerance, e.g., "steroid diabetes," arteriosclerosis, hepatic disease, "alimentary" glycosuria.

Increase in blood pyruvate has been reported in certain cases of decompensated hepatic cirrhosis and hepatic coma. It has been suggested that this may be due to failure of the liver to assimilate pyruvate efficiently into the Krebs cycle sequence of reactions.

GLYCOGEN STORAGE DISEASES

This designation is applied to a group of conditions characterized by an increase in the amount of glycogen, predominantly in the liver and kidneys, but at times also in cardiac and other muscles and in other organs. Four varieties of this condition have been described as entities: (1) Glycogen storage disease of the liver (classical von Gierke's disease); (2) glycogen disease of the heart; (3) galactosemia; and (4) hepatic cirrhosis with abnormal glycogen storage.

Glycogen Storage Disease of Liver (von Gierke). This form exhibits a familial tendency and evidence of disordered carbohydrate metabolism. Glycogen may comprise as much as 16 per cent of the wet weight of the liver, with large deposits also in the kidneys and, to a lesser extent, in other tissues, such as muscle and leukocytes.

Other important biochemical abnormalities are as follows: (1) tendency toward hypoglycemia, more marked with abstention from food; (2) excessive rise in blood sugar after glucose administration; (3) poor glycemic response to epinephrine; (4) hyperlipemia; (5) occasionally, fatty liver; (6) ketosis when food is withheld; (7) increase in blood glycogen; (8) hypoglycemia unresponsiveness in insulin tolerance test (insulin sensitivity).

The underlying abnormality, as in other forms of this condition, appears to be an abnormal stability of the glycogen deposits. Two possible factors have been implicated: (1) formation of a structurally abnormal glycogen, not amenable to degradation by the enzymes normally active in this process (phosphorylase and amyloglucosidase); (2) deficiency in glucose-6-phosphatase, an enzyme necessary for the last step in the formation of glucose from liver glycogen. The latter cannot be implicated in muscle, which does not normally contain this enzyme. Both types of abnormality have been demonstrated. In two fatal cases, the liver contained practically no glucose-6-phosphatase.

Glycogen Disease of the Heart. In this type, the characteristic features are an increase in glycogen in the cardiac muscle and also, frequently, in skeletal and smooth muscle, as well as in parenchymatous organs. The dominant clinical manifestations are referable to the heart and circulation. There are no significant metabolic abnormalities.

Galactosemia. This condition is referred to elsewhere (p. 67). It is characterized by an apparently specific inability to utilize galactose and is accompanied by an excessive amount of glycogen in the liver, the mechanism being unknown.

Cirrhosis with Excessive Glycogen. This form presents the clinical picture of ordinary portal cirrhosis, with hepatomegaly and splenomegaly. There is an excessive amount of glycogen in the liver and in reticuloendothelial structures generally. The glycogen seems to act as a foreign body, inducing a fibrotic reaction wherever it is deposited. The liver glycogen in certain cases has been found to be abnormal in structure. The metabolic abnormalities in this type of glycogen storage disease, when present, are those found in other forms of cirrhosis, and are dependent upon the state of liver function and not on the glycogen abnormality.

EXCRETION OF SUGAR IN URINE

Under ordinary dietary conditions, in normal subjects, glucose is the only sugar present in the free state in the blood plasma in demonstrable amounts. Although normal urine contains virtually no sugar, under certain circumstances glucose or other sugars may be excreted in the urine. This condition may be designated "melituria," the terms glycosuria, fructosuria, galactosuria, lactosuria, and pentosuria being applied specifically to the urinary excretion of glucose, fructose, galactose, lactose, and pentose, respectively. Some employ the term "glycosuria" to indicate the presence of any sugar in the urine, and the term "glucosuria" to indicate the urinary excretion of glucose.

Mechanism of Glycosuria (Glucosuria). Glucose is present in the glomerular filtrate in the same concentration as in the water of the blood plasma (arterial). Under normal conditions it undergoes practically complete reabsorption by the renal tubular epithelial cells and is returned to the blood stream. In normal subjects a very small amount ($<$ 0.5 gm. daily) may escape reabsorption and be excreted in the urine (p. 579). Under controlled conditions, normal kidneys are capable of reabsorbing 250 to 350 mg., and perhaps as much as 450 mg., of glucose per minute. If one assumes an average glomerular filtrate volume of 125 mg. and a maximum arterial blood sugar concentration of about 200 mg./100 cc. (after glucose ingestion), the quantity of glucose delivered to the tubules seldom exceeds 250 mg./min. in normal subjects.

Excretion of abnormal amounts of glucose in the urine may be due to two types of abnormality: (1) increase in the amount entering the tubules per minute; (2) decrease in the glucose-reabsorptive capacity of the tubular epithelium.

(1) The quantity of glucose entering the tubules is the product of (*a*) the minute volume of glomerular filtrate and (*b*) the concentration of glucose in the filtrate, i.e., in the arterial blood plasma. Inasmuch as glomerular filtration is rarely increased markedly, glycosuria of this type is due almost invariably to an increase in the blood sugar concentration above the so-called "threshold" level, i.e., 160–170 mg./100 cc. (venous blood). This may be designated "hyperglycemic glycosuria." It should be pointed out that the amount of glucose presented to the tubules may remain within normal limits in the face of considerably higher blood sugar concentrations if the volume of glomerular filtrate is reduced simultaneously (e.g., glomerular damage complicating diabetes).

(2) Reabsorption of glucose by the renal tubular epithelium is accomplished mainly by an active process, presumably enzymatic, and probably involving phosphorylation. The capacity for reabsorption may be diminished by induced (phlorizin), hereditary ("renal" glycosuria), or acquired (certain types of kidney disease) defects in this specific enzyme mechanism, with consequent glycosuria in the presence of normal or subnormal blood glucose concentrations (non-hyperglycemic glycosuria). These are forms of so-called "renal glycosuria" ("lowered renal threshold" for glucose).

Other Sugars in Urine. The renal tubular epithelial cells are apparently unable to reabsorb significant amounts of sugars other than glucose. Consequently, when these appear in the blood (and, therefore, in the glomerular filtrate) in demonstrable concentrations, they are eliminated in the urine, as indicated elsewhere (p. 65ff.).

ABNORMAL URINE SUGAR

Melituria

The term "melituria" is properly employed to designate the presence, in the urine, of an abnormal amount of sugar. Since all meliturias are not glycosuria, the identification of the sugar present becomes a matter of considerable moment.

Tests for the Detection of Sugars

Metallic Oxide Reduction Tests. The most widely used routine method for the detection of sugar in the urine is one of the copper reduction tests of which the Benedict test is perhaps the most satisfactory. The property of reducing metallic oxides in alkaline solution (copper, bismuth, mercury), possessed by certain sugars, depends upon the presence of a free aldehyde or ketone group in their molecular structure. If Fehling's solution is employed, reduction may be caused by substances other than sugars if present in sufficient concentration. Among these are uric acid, nucleoprotein and conjugate glycuronates formed after the ingestion of antipyrine, menthol, phenol, camphor, chloral, etc. Creatinine may, by dissolving cupric oxide, mask slight degrees of reduction caused by small amounts of sugar. If chloroform is used as a preservative, a falsely positive result may be obtained.

Benedict's test is much more satisfactory than Fehling's. It yields positive results with glucose present in as low a concentration as 0.1 per cent. The

Benedict reagent is furthermore less susceptible to reduction by uric acid and chloroform.

The bismuth reduction test (Nylander) is not commonly employed. It is believed to be capable of detecting smaller quantities of glucose than the Benedict reagent but albumin produces a black color similar to that produced by sugars and so, if present, must be removed before performing the test.

The following sugars are capable of reducing metallic oxides in alkaline solution: glucose, fructose, galactose, pentose, lactose and maltose.

Fermentation Test. The fact that certain sugars are fermentable by yeast has been the basis for the widespread use of the fermentation test in the identification of urinary sugars. The statement is ordinarily made that glucose, fructose and galactose are fermentable by yeast and that maltose and sucrose are fermentable only after their hydrolysis by the enzymes maltase and invertase present in the yeast. Lactose is said to be nonfermentable by ordinary bakers' yeast. One possible source of error has been indicated by Neuberg, who demonstrated that yeast possesses the property of splitting off carbon dioxide from the carboxyl group of amino acids which are normally present in the urine. Another important observation has been made by Castellani and Taylor, who found that ordinary bakers' yeast is not pure and usually consists of one or two species of saccharomyces with a contaminating gram-positive bacillus. They showed that most cultures of so-called "pure yeast" ferment glucose, fructose, galactose, sucrose, maltose and, in many instances (15 per cent), lactose. Obviously, positive differentiation of urinary sugars on the basis of this test is impossible.

Castellani has elaborated a method of differentiating various sugars on the basis of fermentation by specific fungi and gas production by specific bacteria. For example, glucose alone is fermented by Monilia balcanica; glucose and fructose are fermented by Monilia krusei; B. coli forms gas with lactose, whereas B. paratyphosus does not. The reader is referred to the work of Castellani for further details. The combined use of reduction tests and gas production by specific fungi and bacteria is of great value in the positive identification of urinary sugars.

Phenylhydrazine Reaction. This reaction depends upon the formation of a crystalline osazone, the structure of which is typical, to a certain degree, for various sugars. Glucose and levulose form osazone crystals of identical structure. The identification of lactose by this test is not practicable, for lactosazone crystals, although typical, are formed with difficulty in urine. At times the determination of the melting point of these crystals is utilized as a means of differentiating the sugars, but it is not a procedure of practical clinical value.

Specific Rotation. The degree of rotation of polarized light, determined by means of a polariscope or polarizing saccharimeter, may be employed as an aid in the identification of urinary sugars. This procedure is not frequently resorted to clinically. Furthermore, glucose and lactose cannot be differentiated by this method.

Other tests which are of value in the positive identification of urinary sugars will be dealt with in discussing the various types of melituria.

Glycosuria

The term "glycosuria" signifies the excretion, in the urine, of abnormal amounts of glucose. Glucose may be identified in the urine on the basis of the following tests:

(1) Positive reduction test.
(2) Fermentation with bakers' yeast.
(3) Typical glucosazone crystals with phenylhydrazine.
(4) Gas production with Monilia balcanica (Castellani).
(5) Specific rotation of polarized light.

As has been indicated, the properties of reducing power and fermentation by yeast are shared by many sugars and are therefore not specific for glucose. The following criteria may be established for the positive identification of glucose.

(*a*) Typical osazone crystals with phenylhydrazine in the presence of a negative Seliwanoff reaction (resorcinol-hydrochloric acid) to exclude fructose, or

(*b*) Gas production with Monilia balcanica (Castellani), or

(*c*) Specific rotation of polarized light (+52.5°) in the absence of a positive mucic acid test to exclude lactose.

As has been previously indicated, the several forms of glycosuria may be conveniently classified clinically under two headings:

1. Glycosuria unassociated with hyperglycemia.
2. Glycosuria associated with hyperglycemia.

Nonhyperglycemic Glycosuria. Glucose may appear in the urine in the presence of a normal concentration of sugar in the blood. This condition may be produced experimentally by the administration of the glucoside phlorizin. It is observed clinically in so-called "renal glycosuria" (renal diabetes), during pregnancy, and, as some believe, in the condition commonly termed "alimentary glycosuria."

PHLORIZIN GLYCOSURIA. The administration of phlorizin, orally or, better, subcutaneously, is followed by glycosuria associated with a normal, and indeed, in many instances, a subnormal blood sugar concentration. The theoretical aspects of this interesting and physiologically important condition cannot be dwelt upon except as they serve to throw light upon the possible existence of forms of glycosuria observed clinically, dependent upon factors operating locally in the kidneys. There appears to be little doubt that phlorizin glycosuria is in the true sense of the term a renal glycosuria. There is rather convincing experimental evidence which suggests that this substance produces it glycosuric effect by inhibiting reabsorption of glucose from the renal tubules, probably by inhibiting phosphorylation (hexose-phosphate formation) (p. 2).

RENAL GLYCOSURIA (RENAL DIABETES). The frequency of incidence of this condition, also known as "benign" glycosuria and "diabetes innocens," is perhaps not as great as is commonly supposed. When rigid diagnostic criteria are applied, the reported incidence is usually less than 1 per cent of cases of glycosuria, although this condition has been reported as comprising 9 to 26 per cent of all cases of glycosuria in army recruits. The following standards have been set for the diagnosis of true renal glycosuria:

(1) Fasting blood sugar within normal limits and a normal or supernormal flat glucose tolerance curve.

(2) Glucose should be present in every specimen of urine, whether voided in the fasting state or after a meal. The quantity of sugar in the urine should be largely independent of the diet, although it may vary somewhat depending on the amount of carbohydrate ingested.

(3) Carbohydrate utilization should be normal, as evidenced by determinations of respiratory quotient and serum inorganic phosphorus after glucose ingestion.

(4) There should be no disturbance of fat metabolism, ketosis being more likely to develop when the patient fasts than when he overeats.

(5) Moderate doses of insulin should have little or no effect upon the glycosuria.

This condition is believed by many to be hereditary and familial, and it seems likely that, once developed, it persists throughout the life of the individual. The importance of its recognition depends upon its apparent harmlessness; so far as can be determined, it never results in diabetes mellitus or in any metabolic derangement whatsoever. Practical danger in making the diagnosis of renal glycosuria lies in the confusion of this condition with potential or mild diabetes mellitus. The patient must be observed carefully over a prolonged period before the diagnosis of renal glycosuria can be established definitely.

The essential cause is unknown. Whatever it may be, inefficiency of the mechanism for tubular reabsorption of glucose is the fundamental defect that determines the occurrence of glycosuria in this condition. From this standpoint it resembles phlorizin glycosuria. No metabolic disturbance occurs in subjects with renal glycosuria as long as the carbohydrate intake is adequate to compensate for the amount lost in the urine. Deprivation of carbohydrate may cause hypoglycemia, increased sensitivity to insulin, and ketosis more readily than in normal subjects.

FANCONI SYNDROME (p. 183). This term is applied to a usually more extensive type of renal tubular dysfunction, usually occurring in children, in which there is defective reabsorption of amino acids, other organic acids, and phosphate, as well as glucose. It is presumably due to a developmental defect of the tubules. A perhaps related type of renal glycosuria occurs occasionally in hepatolenticular degeneration (Wilson's disease), which is also characterized by aminoaciduria.

GLYCOSURIA OF PREGNANCY. Glycosuria may occur in 15 to 25 per cent of all apparently normal pregnant women, particularly in the later months and more frequently in primigravidae than multigravidae. Some reports state the incidence to be as high as 35 to 60 per cent, but these figures must be accepted with caution. Many have regarded this as a form of renal glycosuria, since the fasting blood sugar is not elevated. However, more extensive use of glucose tolerance tests has provided data which suggest that decreased glucose tolerance, perhaps physiological, may form the basis for the glycosuria of normal pregnancy (p. 47). Great care must always be exercised to exclude the possibility of latent diabetes mellitus. It must also be borne in mind that lactose, which gives a positive reduction test, may be present in the urine late in pregnancy, although usually in small amounts, and should not be mistaken for glucose (p. 67).

"ALIMENTARY" GLYCOSURIA. Opinion is divided regarding the metabolic status of so-called "alimentary" glycosuria. The term is employed to designate the urinary excretion of glucose by certain apparently normal individuals after the ingestion of excessive amounts of cane sugar, glucose or, at times, starch. The occurrence of glycosuria under such circumstances may be due either to a "lowering of the renal threshold" for glucose or to the absorption of glucose from the intestine at a rate too rapid to allow of its adequate removal from the circulation by the liver (e.g., in hyperthyroidism).

Woodyatt and his associates have shown that the normal individual can utilize glucose injected intravenously in amounts up to 0.8 gm. per kilogram of body weight per hour; when this rate is exceeded glycosuria occurs. It has also been demonstrated that the absorption of glucose from the intestine proceeds normally at a maximum rate of 1.8 gm. per kilogram of body weight per hour, regardless, within wide limits, of the quantity of sugar ingested. Consequently, if the liver removes a minimum of 1.0 gm. per kilogram per hour, allowing 0.8 gm. to pass into the general circulation, glycosuria should not be expected to occur in normal individuals. In the absence of any abnormality of hepatic or tissue glycogenic function, alimentary glycosuria might be explained upon the basis of increased permeability of the intestinal mucosa for glucose, resulting in its absorption at a rate more rapid than can be adequately handled by the liver; it therefore reaches the tissues, including the kidneys, in excessive amount, with the result that a portion is eliminated in the urine. This hypothesis does not necessarily imply the existence of venous hyperglycemia, for, tissue utilization being unimpaired, slight grades of arterial hyperglycemia may possibly be corrected and the concentration of glucose in the blood leaving the tissues may be within normal limits. This possibility has been supported by studies of capillary and venous blood sugar tolerance curves in benign glycosuria. Some authorities believe that alimentary glycosuria, in most instances, is in reality renal glycosuria. Others maintain that many such cases are dependent upon some disturbance of intermediary carbohydrate metabolism originating in the liver, endocrine glands or tissues (muscles). No such disturbance can be demonstrated satisfactorily in most cases.

The capacity of the normal organism for metabolizing or holding sugar may be influenced by diet. In an individual whose habitual carbohydrate intake is small, or who has been subjected to carbohydrate restriction, the ingestion of an excessive quantity may result in glycosuria. This is due to an excessively high rise in blood sugar concentration, the so-called "Staub-Traugott effect" (p. 28).

GLYCOSURIA IN GLOMERULONEPHRITIS AND NEPHROSIS. Glycosuria occurs at times in a considerable proportion of patients with glomerulonephritis, nephrosclerosis and nephrosis. In some with glomerulonephritis and nephrosclerosis, glycosuria is associated with fasting hyperglycemia or diminished glucose tolerance or both (see pp. 38, 47). However, in many such cases and in individuals with nephrosis, the glycosuria appears to be dependent upon failure of the renal tubules to reabsorb glucose completely from the glomerular filtrate as a result of the degenerative changes in the renal tubular epithelium. In such cases the urine may contain more than 1 per cent of glucose after the ingestion of a carbohydrate-rich meal (p. 580). The probable reason for the inconsistent occurrence of glycosuria in glomerulonephritis is the fact that the glucose load delivered to the tubules, i.e., the amount filtering through the glomeruli per minute, is decreased as a result of glomerular damage, and may therefore remain within the reabsorptive capacity of the damaged tubular epithelium.

Hyperglycemic Glycosuria. The occurrence of glycosuria in association with hyperglycemia is readily understood. If one accepts the normal "renal threshold" value as being about 160 mg. of glucose per 100 cc. of blood, the elimination of glucose in the urine may be expected in the presence of higher blood sugar levels. The fact must be kept in mind, however, that the renal threshold may exhibit rather wide variations in different individuals and under different conditions. Obviously, the causes of hyperglycemia are potential causes of glycosuria. These include the following:

DIABETES MELLITUS (p. 71). Diabetes mellitus is the most frequently observed individual cause of glycosuria dependent upon hyperglycemia. The view that glycosuria always indicates diabetes mellitus is, however, erroneous, even in the presence of hyperglycemia. Studies of large series of patients with glycosuria have revealed diabetes to be the cause in about 40 per cent of cases, the incidence falling to about 30 per cent in glycosuric subjects under 20 years of age. As has been mentioned previously, glycosuria is not uncommon in the later months of pregnancy. Its occurrence in the early months should lead to a rigid investigation of the possibility of the existence of diabetes mellitus. The differential diagnosis between diabetes and other conditions causing hyperglycemic glycosuria entails an investigation of blood sugar tolerance curves, the arterial-venous blood sugar difference, the serum phosphate curve and respiratory quotient variations following glucose ingestion, ketosis, acid-base balance, the plasma cholesterol concentration, and the basal metabolic rate (hyperthyroidism). Further details will be considered in the section on diabetes mellitus (p. 73).

HYPERTHYROIDISM. Glycosuria occurs in 25 to 35 per cent of patients with hyperthyroidism. Its incidence is somewhat higher in cases of primary (exophthalmic goiter) than of secondary hyperthyroidism (toxic adenoma). Associated with manifestations of hyperthyroidism, it is usually readily distinguishable from other causes of glycosuria and hyperglycemia. It must be recognized that diabetes and hyperthyroidism may coexist and that hyperthyroidism is likely to act as a predisposing cause of diabetes. Glycosuria in this condition may be due in part to an increased rate of absorption of glucose from the intestine (alimentary glycosuria) as well as to lowered tolerance.

HYPERPITUITARISM. Glycosuria occurs in 25 to 40 per cent of patients with acromegaly at some time during the cause of the disease. It occurs also as a part of the characteristic clinical picture of pituitary basophilism (Cushing's syndrome), but is not present in all cases.

ADRENAL HYPERFUNCTION. Glycosuria may occur during periods of paroxysmal hypertension and hyperglycemia in patients with adrenal medullary tumor (pheochromocytoma), due to increased epinephrine secretion. It is also a feature of the clinical picture of Cushing's syndrome due to adrenal cortical hyperplasia or tumor.

EMOTIONAL GLYCOSURIA. Glycosuria may occur during periods of excessive nervous strain and emotional excitement. This condition has been termed "emotional" or "psychic" glycosuria, and has been attributed to increased secretion of epinephrine (p. 35). It has been found in normal subjects exposed to the emotional excitement and worry of athletic contest, anticipated operative procedure, and college and insurance examinations. Its incidence in college students under the stress of final examinations has been reported as 1.6 per cent.

MISCELLANEOUS. Glycosuria may occur in hemochromatosis, advanced pancreatitis, and severe hepatocellular damage, in the latter usually with a normal fasting blood sugar concentration and only after a high carbohydrate intake. It is seen at times with head injuries or other causes of increased intracranial pressure (brain tumor, abscess, hemorrhage), hypothalamic lesions, ether or chloroform anesthesia, narcosis by morphine or barbiturates, asphyxia, acidosis, acute and chronic infections, coronary artery occlusion, advanced malignancy, simple obesity, and essential hypertension. It has also been observed after administration of caffeine, diuretin, strychnine, bichloride of mercury, and chromates. In these conditions, the persistence and severity of glycosuria are related usually to the persistence and severity of the accompanying hyperglycemia (pp. 36–38), except in the presence of renal tubular damage. Consequently, it is usually transitory and of mild degree (less than 1 per cent) in conditions other than diabetes mellitus, hemochromatosis, and Cushing's syndrome.

Fructosuria

Fructose reduces metallic oxides in alkaline solution, is fermentable by bakers' yeast and yields an osazone with phenylhydrazine which is morphologically identical with glucosazone. Fructose may be identified in the urine by the following methods:

(1) Gas production with Monilia krusei but not with Monilia balcanica, to exclude glucose.

(2) Characteristic osazone crystals and positive Seliwanoff (resorcinol-HCl) or Borchardt reaction, to exclude glucose. The presence of nitrites or indican in excess interferes with the development of the characteristic yellow color of Borchardt's reaction. Glucose, in large amount (2 per cent), may yield a positive Seliwanoff reaction.

(3) Rotation of polarized light to the left in the absence of other levorotatory substances, as conjugate glycuronates and betahydroxybutyric acid.

Fructose may appear in the urine under the following circumstances:

(*a*) In severe cases of diabetes mellitus, always in association with glucose.

(*b*) Alimentary fructosuria, following the ingestion of large quantities of fructose, particularly in patients with hepatic insufficiency. This has been utilized as a test of hepatic function but is unsatisfactory; approximately 10 per cent of normal individuals eliminate fructose in the urine following the ingestion of 100 gm. of fructose.

(*c*) Essential fructosuria, implying the occurrence of fructosuria in the absence of the above-mentioned factors, is a rare condition. Some regard it as a specific, probably inborn error of metabolism, localized primarily in the liver where a specific enzyme deficiency exists, resulting in impaired ability to fix fructose as glycogen. The metabolism of other carbohydrates is undisturbed. A few cases have been reported in which there was a total absence of tolerance for fructose, the sugar being eliminated if any whatsoever was ingested. Insulin has no influence upon this condition; no rise in the respiratory quotient follows the administration of fructose to such individuals, indicating failure of utilization of that sugar. It is said that rectal administration produces more severe fructosuria than when given by mouth.

Pentosuria

Pentoses reduce metallic oxides in alkaline solution and are non-fermentable by bakers' yeast. They may be identified in the urine by the following methods:

(1) Positive Bial Reaction (orcinol-HCl).

(2) Positive Benedict reaction and negative fermentation test with bakers' yeast in the absence of lactose and nonsugar reducing substances (conjugate glycuronates).

(3) Characteristic pentosazone crystals with phenylhydrazine.

(4) Positive Benedict reaction, gas production with Bacillus coli communis and B. paratyphosus B (to exclude lactose), and no fermentation by Monilia tropicalis (to exclude galactose).

Pentose may appear in the urine under the following circumstances:

(*a*) Alimentary pentosuria.

This is a temporary condition, occurring in normal individuals after the ingestion of large quantities of fruits which have a high pentose content (prunes, cherries, grapes, plums). It is of no clinical significance apart from the fact that it may be mistaken for glycosuria because of a positive copper-reduction test. The urinary pentose in this condition is L-arabinose.

(*b*) Diabetes mellitus (some cases).

(*c*) Essential pentosuria (chronic pentosuria).

This is a relatively rare and extremely interesting condition which is analogous to essential fructosuria. Pentose is more or less constantly present in the urine, the quantity excreted bearing no relation to the amount ingested. It is of no known clinical significance, since the utilization of other carbohydrates is unimpaired. It appears to be familial and hereditary in nature, occurring practically exclusively in Jewish subjects, predominantly in males. As in the case of alimentary pentosuria, its chief importance lies in the possibility of mistaking it for glycosuria. The urinary pentose in this condition is L-ketoxylose.

Lactosuria

Lactose reduces metallic oxides in alkaline solution (Benedict, Fehling, etc.) and, in about 15 per cent of cases, is fermented by bakers' yeast. It may be identified in the urine by the following methods:

(1) Positive Benedict test, gas production with B. coli communis and no gas production with B. paratyphosus B (to exclude pentose).

(2) Positive mucic acid test and negative phloroglucinol-HCl reaction (Tollens) to exclude galactose.

(3) Characteristic lactosazone crystals with phenylhydrazine. This test is usually unsatisfactory.

(4) If positive Benedict test and no fermentation with bakers' yeast, negative Bial test to exclude pentose.

Lactosuria occurs in a considerable proportion of women during the period of lactation. Although this condition has generally been regarded as being confined to the puerperium, recent studies indicate that small amounts of lactose may be found in the urine in about 50 per cent of all pregnant women, the incidence increasing as pregnancy advances. It appears more frequently in the afternoon, and in primigravidae.

Galactosuria

Galactose reduces metallic oxides in alkaline solution and is fermented by most samples of bakers' yeast, although usually not as actively as are glucose and fructose. It may be identified by the following methods:

(1) Positive mucic acid test to exclude all other reducing substances except lactose, and positive phloroglucinol-HCl reaction (Tollens) to exclude lactose.

(2) Positive Tollens reaction and no absorption bands upon spectroscopic examination (to exclude pentose and glycuronic acid).

Galactosuria is not frequently observed except following the ingestion of supertolerance doses of galactose (p. 30). It has been found to occur in nursing infants in association with derangements of gastrointestinal function (from lactose) and, also, in both infants and adults with hepatic disease. A condition of galactemia has been described in infants exhibiting the following characteristics: (*a*) athrepsia with insatiable hunger; (*b*) hepatomegaly (increased liver glycogen); (*c*) lamellar cataracts; (*d*) galactosuria; occasionally (*e*) mental retardation.

Maltosuria

This is a rare condition, of no apparent clinical significance.

DIABETES MELLITUS

According to the classical concept, excluding the relatively few instances secondary to demonstrable pituitary or adrenal cortical hyperfunction (e.g., acromegaly, Cushing's syndrome, adrenal cortical tumor or hyperplasia), clinical diabetes mellitus is regarded as due to disease of the pancreatic islets resulting in deficient secretion of insulin. The anatomical pancreatic causes of insulin deficiency have been classified as follows:

- I. Destruction of pancreatic tissue (islet and acinar).
    - (a) Pancreatitis, acute and chronic.
    - (b) Malignancy of pancreas.
    - (c) Hemochromatosis.
    - (d) Toxic injury.
- II. Selective destruction of islet cells.
    - (a) Hyalinization.
    - (b) Fibrosis.
    - (c) Toxic injury and lymphocytic infiltration.
    - (d) Hydropic degeneration.
- III. Congenital inadequacy of islet cells.
- IV. Inadequate blood supply (arteriosclerosis).

Experimental demonstration of the influence of the pituitary and adrenal glands, the liver and other extrapancreatic tissues upon carbohydrate metabolism has led to attempts to involve these organs in the etiology and pathogenesis of clinical diabetes mellitus. Among the hypotheses that have been advanced may be mentioned the following: (1) that diabetes is due in some instances to excessive secretion of the "diabetogenic hormone" of the anterior hypophysis; (2) that it may be dependent upon imperfect inhibition of excessive glycogenolysis and excessive gluconeogenesis; (3) that it may be due in some instances to deficient formation of an hypothetical insulin-activator or insulin kinase, secreted perhaps by the liver or duodenum. Although there is no direct evidence of the validity of such hypotheses, the data at hand suggest the participation of some extra-insular factor in the etiology of some cases of clinical diabetes mellitus, and the view is constantly becoming more definitely justified that this condition may have its fundamental origin in something other than a mere state of hypoinsulinism.

Experimental Diabetes Mellitus

Metabolic counterparts of clinical diabetes mellitus can be produced experimentally by disturbing the balance between insulin, on the one hand, and adrenocortical and anterior pituitary hormones on the other (p. 15). This induced imbalance is in the direction of a decrease in the amount of insulin in relation to that of the other two types of hormones. The importance of the balance between these fundamental regulatory factors is reflected in the following facts: (1) the typical diabetic state, with minor differences, results from either (*a*) absolute deficiency in insulin or (*b*) absolute excess of anterior pituitary or adrenocortical hormones (i.e., relative deficiency in insulin); (2) the severe diabetes produced by total pancreatectomy is alleviated strikingly by subsequent removal of either the anterior pituitary or the adrenals.

Total Pancreatectomy. Surgical removal of all pancreatic tissue is followed by characteristic manifestations of diabetes mellitus, the severity of which varies considerably in different species (e.g., severe in dog and cat; moderately severe in man; mild in monkey, goat, pig; none in duck). Removal of the sources of insulin (islet β-cells) (in susceptible species) results in the following metabolic phenomena.

(1) Decreased utilization of glucose, indicated by: (a) decreased glucose tolerance (blood sugar curve) (p. 43); decreased R.Q. (p. 32); fasting hyperglycemia (p. 32); glycosuria (p. 64).

(2) Increased gluconeogenesis (from protein), indicated by negative nitrogen balance and increasing urinary glucose: nitrogen (G:N) ratio. This is due to relative preponderance of protein catabolic adrenocortical hormones (p. 000).

(3) Increased mobilization of body lipids, due to relative preponderance of pituitary and adrenocortical hormones (p. 91). This is reflected in loss of body fat and increase in blood plasma lipids.

(4) Increased oxidation (in liver) of the fatty acids, mobilized in excess, to active two-carbon fragments (p. 90). These cannot be utilized adequately for lipogenesis in the absence of insulin, and are present in amounts greater than can be oxidized in the liver (tricarboxylic acid cycle). They are therefore diverted in increased amounts to the formation of acetoacetate and cholesterol. This results in ketosis, ketonuria and hypercholesterolemia.

(5) The excessive production of acetoacetic and β-hydroxybutyric acids results in acidosis, due to primary reduction in bicarbonate (p. 75).

(6) Dehydration, initiated by the polyuria incident to the glycosuria. Excessive amounts of Na and K are lost from the organism, in addition to water. In advanced stages, e.g., in diabetic coma, the plasma volume decreases, and shock and renal failure develop.

In man and the dog, the diabetes produced by removal of 90 to 95 per cent of the pancreas is more severe than that which follows total pancreatectomy. This has been attributed to the aggravating influence, in the former case, of the hyperglycemic factor, presumably secreted by the α-cells of the pancreatic islets. The residual β-cells undergo degeneration as a result of the prolonged hyperglycemia, as indicated below.

Subtotal Pancreatectomy. In certain species, if an amount of pancreatic tissue inadequate to produce diabetes is removed and the blood sugar is maintained subsequently at a high level by various means, diabetes ensues. At first this is mild and reversible; later it is severe and irreversible. The hyperglycemia may be induced by intraperitoneal administration of glucose, administration of a high caloric diet, Bernard puncture (p. 35), or anterior pituitary extracts (p. 16).

The elevated blood sugar constitutes a stimulus to the remaining β-cells, which, if the hyperglycemia continues, presumably pass through phases of hyperfunction, functional exhaustion, vacuolization, and other degenerative changes eventuating in hyalinization or fibrosis, i.e., permanent destruction. The importance of hyperglycemia in inducing morphological damage of the β-cells is indicated by the fact that these lesions do not develop if the elevation of blood sugar is prevented by simultaneous administration of insulin or phlorizin.

Alloxan Administration. Injection of alloxan, a substance related to the pyrimidine bases and to uric acid, causes permanent diabetes, after brief transitory phases of hyper- and hypoglycemia. The diabetes is due to degeneration and resorption of the β-cells of the pancreatic islets, the α-cells and acinar tissue being unaffected. The alloxan acts directly, promptly, and specifically on the β-cells, and its effect can be prevented by administration of cysteine, glutathione, BAL (dimercaptopropanol), or thioglycollic acid immediately before or within a few minutes after injection of the alloxan. This protective action is due apparently to the —SH (sulfhydryl) content of these compounds, the alloxan being perhaps reduced to an inactive substance. A similar diabetogenic effect is produced by dehydroascorbic and dehydroisoascorbic acids, which resemble alloxan structurally.

There are interesting differences between the diabetes that follows pancreatectomy and that produced by alloxan. In alloxan diabetes, hyperglycemia and glycosuria are more severe and the insulin requirement is higher, but the animals survive longer without insulin than do depancreatized animals, show little ketonuria, and do not go into coma. If alloxan diabetic dogs are subsequently depancreatized, glycosuria and the insulin requirement diminish but ketosis and coma supervene rapidly if insulin is withheld. It has been suggested that the differences between the two diabetic states are dependent upon a hormone elaborated by α-cells (hyperglycemic factor?), present in the alloxanized but not, of course, in the depancreatized animal.

Administration of Adrenocortical Hormones. The effects of the 11-oxygenated adrenal hormones on carbohydrate metabolism are discussed elsewhere (p. 16). Diabetes develops in subjects with adrenocortical hyperfunction and can be induced in rats by administration of these hormones together with a high carbohydrate diet. There is a considerable species difference in this regard. A temporary diabetic state occurs at times, but not invariably, in man following prolonged administration of adrenal oxysteroids or ACTH. "Adrenal diabetes" differs from "pancreatic diabetes" chiefly in the associated increased resistance to insulin and the rather consistent increase in liver glycogen.

Anterior Pituitary Extracts. The influence of hormones of the anterior pituitary on carbohydrate metabolism is described elsewhere (p. 16). Injection of crude saline anterior pituitary extracts (APE) causes hyperglycemia and, if repeated over periods of 1½ to 4 weeks, permanent diabetes in certain species, e.g., adult dog and cat. The rabbit is moderately susceptible, but the rat and mouse are highly resistant to this influence. The permanent diabetes so induced is due to destruction of the islet β-cells induced by the prolonged hyperglycemia. It does not develop if the latter is prevented by simultaneous (with APE) administration of insulin.

This "diabetogenic" action of anterior pituitary extracts is not due entirely to ACTH, for it occurs in adrenalectomized animals. It has been attributed to the pituitary growth hormone or to some principle intimately associated with it (p. 16).

Thyroid Administration. Diabetes ("metathyroid diabetes") can be produced in partially pancreatectomized dogs in which hyperglycemia is maintained by administration of thyroid hormone. The islet β-cells undergo degeneration, as described above with hyperglycemia induced by other means.

Carbohydrate Metabolism in Hypophysectomized-Depancreatized Animals. Houssay demonstrated that manifestations of diabetes in depancreatized animals are prevented or alleviated by hypophysectomy. Such animals ("Houssay animals") differ from those merely depancreatized as follows:

(1) Glycosuria and polyuria are diminished and at times absent.

(2) They survive for longer periods, with less weight loss, are less susceptible to infection, and wounds tend to heal more rapidly.

(3) The blood sugar is maintained at lower levels and is occasionally subnormal.

(4) The Houssay animal is extremely sensitive to insulin, as is the simply hypophysectomized animal.

(5) There is less ketosis, and the plasma bicarbonate remains within normal limits.

(6) Hepatic and muscle glycogen may be normal and the R.Q. may exhibit an almost normal increase following ingestion of glucose.

(7) The glucose tolerance curve in some cases is less distinctly abnormal, although it usually remains of the "diabetic" type.

(8) The nitrogen balance becomes less markedly negative.

These animals are, however, far from normal. They are rather precariously balanced between hypoglycemia and hyperglycemia. The blood sugar exhibits wide fluctuations, responding unduly to abstinence from (hypoglycemia) and administration of (hyperglycemia) carbohydrate. The ameliorating influence of hypophysectomy on pancreatic diabetes is apparently due to diminished gluconeogenesis and ketogenesis, and improved utilization of glucose at a more normal blood sugar concentration. Similar improvement occurs in depancreatized animals if the adrenals are removed, and not the pituitary. These observations emphasize the physiological importance in this connection of the balance between insulin, on the one hand, and hormones of the anterior pituitary and adrenal cortex, on the other.

Clinical Diabetes Mellitus

Despite the lack of exact knowledge regarding the pathogenesis of clinical diabetes mellitus, considerable information is available concerning the pathologic physiology of this condition. Perhaps in no other clinical disorder is an understanding of the metabolic manifestations of the disease process so essential to accurate diagnosis and proper treatment. The important fundamental metabolic aberrations will therefore be considered in some detail.

Hyperglycemia. Fasting hyperglycemia is one of the most important diagnostic features of diabetes mellitus. It occurs as a result of the fact that the rate of withdrawal of glucose from the blood is diminished because of the impaired ability of the tissues to store glycogen and utilize glucose. The degree of hyperglycemia is not, however, an accurate index of the severity of the condition because of the influence of such added factors as age, the state of nutrition, the available carbohydrate supply, the degree of acidosis, the presence of infection and the state of hepatic and renal function. In mild cases the fasting blood sugar may be within normal limits (65 to 110 mg. per 100 cc.), the existing disturbance of carbohydrate metabolism being evidenced only by the application of such procedures as the determination of the tolerance to glucose or the respiratory quotient. In moderately severe cases values as high as 300 mg. per 100 cc. may be obtained. In severe cases the fasting blood sugar level may rise to 600 mg. per 100 cc. or higher, values as high as 2000 mg. having been reported.

There is a remarkably wide range of variability in blood sugar values in patients in diabetic coma. This condition has been present with blood sugar concentrations as low as 130 mg. per 100 cc. and has been absent with values as high as 1500 mg. per 100 cc. Coma is most likely to develop in the presence of relatively low blood sugar values in patients below the age of twelve years and above the age of fifty years, and in those with acute and overwhelming infection. Moreover, there appears to be no consistent relationship between the blood sugar concentration and the occurrence of death or recovery in patients with diabetic coma. Death may occur with values below 300 mg. per 100 cc. and recovery has been reported in patients with blood sugar concentrations as high as 1800 mg. per 100 cc. Obviously, the blood sugar concentration alone is not a reliable criterion for judging the severity of diabetic coma in any individual case.

Decreased Carbohydrate Tolerance (see p. 43). Diminution in the tolerance of the organism to glucose ranks in importance with decrease in the respiratory quotient in the diagnosis of diabetes mellitus. It is of particular value, clinically, in those cases in which the fasting blood sugar is within normal limits. A diagnosis of diabetes mellitus cannot be made in the absence of a decrease in the carbohydrate tolerance of the organism. The abnormally high and prolonged blood sugar curve following the ingestion of glucose (p. 44) is characteristically associated with a diminished arterial-venous blood sugar difference and with a diminution in the degree to which the blood inorganic phosphate falls during the period of increased carbohydrate supply. These phenomena are generally interpreted as indicating diminished tissue utilization of glucose. The glucose tolerance test is useful only in mild or moderately severe cases of diabetes mellitus where the diagnosis may be in doubt, but it should not be applied in severe cases.

As stated elsewhere (p. 68), the belief that abnormalities in the glucose tolerance curve in patients with diabetes mellitus are indicative of a diminished quantity or activity of insulin, or that they can be interpreted in terms of diminished utilization of glucose, has been subjected to considerable opposition in recent years. Some believe that the excessively high and prolonged tolerance curve is more directly dependent upon excessive gluconeogenesis and glycogenolysis in the liver than upon impaired glucose utilization in the tissues. Moreover, observations upon depancreatized animals that have been also hypophysectomized or adrenalectomized indicate clearly that the organism is capable of utilizing glucose in the absence of insulin.

These two views may be expressed as follows:

(1) NONUTILIZATION THEORY. In the absence of insulin, the capacity of the peripheral tissues to metabolize glucose is greatly diminished. Moreover, the anterior pituitary and adrenal cortex, unopposed by insulin, stimulate gluconeogenesis in the liver and further depress utilization of glucose.

(2) OVERPRODUCTION THEORY. This postulates that utilization of glucose in the peripheral tissues is not influenced greatly by insulin, the anterior pituitary, or the adrenal cortex. It maintains that the fundamental defect is stimulation of gluconeogenesis in the liver, from amino acids (protein catabolism), by the anterior pituitary and adrenal cortex in the absence of adequate amounts of insulin. It is pertinent in this connection that insulin has been shown to decrease the output of glucose from the liver.

There seems little doubt that, in the intact organism, insulin facilitates utilization of glucose. Its effect in counteracting pituitary inhibition of the hexokinase reaction has been mentioned elsewhere (p. 15). It appears likely that the metabolic derangement in diabetes mellitus is based upon both underutilization and overproduction of glucose.

However, whether one subscribes to the overproduction or underutilization theory, clinical experience has demonstrated the practical diagnostic importance of the glucose tolerance test. Some authorities regard a blood sugar concentration of 170 mg. per 100 cc. or more following a meal as indicative of diabetes mellitus. If capillary blood is used for the sugar determination the critical diagnostic level is raised to 200 mg. per 100 cc. Delay in the return to an approximately normal level within two hours is usually more significant in this connection than is the magnitude of the initial rise (p. 44).

Glycosuria (see p. 64). Glucose is eliminated in the urine when the blood sugar level rises above the "renal threshold" for glucose which, under normal conditions, ranges from 160 to 180 mg. per 100 cc. Since in mild cases of diabetes the fasting blood sugar level may be below this point, glycosuria may occur intermittently, chiefly following the ingestion of carbohydrate-rich meals. Under such circumstances it is obvious that single specimens of urine may not contain glucose and it is important that the examination be conducted on twenty-four-hour specimens of urine or on all voided specimens. In more severe cases, the blood sugar being maintained at a relatively high level, glycosuria of varying degree occurs continuously. In mild cases the urine (twenty-four-hour sample) may contain from a trace to 1 per cent of glucose; moderately severe cases, 1 to 3 per cent; severe cases, 3 to 8 per cent. Concentrations higher than 8 per cent are rarely observed because of the difficulty of maintaining larger amounts of glucose in solution in the urine. The kidney responds to the necessity for the elimination of larger quantities of glucose by the simultaneous excretion of increased quantities of water. Polyuria is almost invariably observed as the concentration of glucose in the urine rises above 4 per cent. Under such circumstances, if water is supplied in abundance, extremely large volumes of urine may be passed daily, occasionally as much as 2 or 3 gallons. The elimination of such large quantities of water frequently results in extreme grades of dehydration. One of the characteristic features of the polyuria of diabetes mellitus is the high specific gravity of the urine, frequently 1.030 or more, which is due to its high sugar content. This feature distinguishes it from polyuria due to other conditions in which the specific gravity of the urine is usually in inverse proportion to the urine volume.

In some cases glycosuria may be absent although the blood sugar concentration is above the normal "renal threshold" level (see p. 58) and, at times, may be extremely high. The occurrence of hyperglycemia without glycosuria is ascribed to an increase in the "renal threshold" for sugar and is observed most commonly in association with arteriosclerosis or glomerulosclerosis in elderly individuals with diabetes of long standing. Sudden, marked variations in the "renal threshold" for sugar may occur in such individuals.

Decreased Respiratory Quotient. The respiratory quotient is an index of the relative percentages of fat, protein and carbohydrate oxidized in the organism during the experimental period (see p. 366). The R.Q. for carbohydrate being 1.0, that for protein being 0.8 and for fat 0.7, it is generally assumed that a decrease in the respiratory quotient from the value characteristic of normal subjects on a mixed diet under resting conditions, 0.85, approaching 0.7 in complete diabetes, is indicative of the degree of impairment of carbohydrate utilization in this condition. Muscular exercise, epinephrine, and glucose, which cause a rise in the respiratory quotient of normal individuals, usually have no such effect in untreated diabetic patients. In some instances there may actually be a diminution in the respiratory quotient following the ingestion of glucose. Certain factors, such as the general state of nutrition and the degree of acidosis, exert an influence upon the respiratory quotient values, which, however, constitute perhaps the most satisfactory index of the severity of the condition, improvement being indicated by a rise in the respiratory quotient. It should be mentioned here that some authorities believe that overproduction of glucose (from amino acids) rather than impaired utilization of glucose constitutes the fundamental abnormality of carbohydrate metabolism in diabetes mellitus. The R.Q. for the conversion of protein to glucose has been estimated as 0.632 to 0.706, that for gluconeogenesis from fat (?) being about 0.281. According to the proponents of the overproduction theory, the usual diabetic R.Q., approaching 0.7, is regarded as the resultant of two processes proceeding simultaneously, (*a*) gluconeogenesis from protein and fat (?), with an R.Q. ranging from 0.2 to 0.7 and (*b*) the oxidation of carbohydrate, with an R.Q. of 1.0.

Lipemia and Ketosis (pp. 95, 113). As the impairment of carbohydrate utilization progresses in diabetes, the metabolism of fat is proportionately increased in order to maintain the energy requirements of the organism. This increase in the intermediary metabolism of fat is accompanied by an increase in the concentration of lipids in the blood. The blood plasma normally contains 140 to 260 mg. of cholesterol per 100 cc., 110 to 485 mg. of fatty acids, 0 to 260 mg. of neutral fat and 110 to 250 mg. of phospholipid. Values for total blood fat as high as 25 per cent have been observed; figures above 10 per cent are extremely unusual. From a clinical standpoint the degree of hypercholesterolemia may be accepted as usually indicative of the degree of increase in the total lipid concentration in diabetes. Although there is no definite parallelism between cholesterolemia and glycemia, glycosuria, ketonuria or acidosis, many believe that, particularly in untreated cases, the degree of cholesterolemia is an important index of the severity of the diabetic condition. Plasma cholesterol values above 1000 mg. per 100 cc. are occasionally observed, the usual range being from 250 to 400 mg. per 100 cc. In many cases the plasma cholesterol remains above normal for varying periods of time after the blood sugar has fallen to within normal limits. It therefore serves as a valuable measure of the efficacy of therapeutic procedures and as an indication of the necessity for further treatment. It must be kept in mind that a drop in plasma cholesterol may occur in severe forms of the disease, particularly in terminal states. The cause of this phenomenon is not known (p. 122).

The increase in neutral fat in the plasma is due mainly to excessive mobilization from the fat depots (adipose tissue), stimulated by the hormonal imbalance in the direction of a relative excess of adrenocortical hormones and growth hormone (p. 91). Fatty liver may develop (p. 106). This same imbalance results in increased catabolism of fatty acids, with the consequent production of an excess of "acetate" molecules. In the presence of insulin deficiency, these cannot be incorporated adequately in the tricarboxylic acid cycle (oxalacetate deficiency, p. 8), nor can they be utilized adequately for fatty acid synthesis (p. 90). Consequently, in the liver, they are diverted, in excess, into plasma cholesterol synthesis (p. 114), with consequent hypercholesterolemia, and into ketogenesis (p. 95), with consequent ketosis (p. 95). The possible relation of the hypercholesterolemia to the development of atherosclerosis is discussed elsewhere (p. 118).

The disturbance in lipid metabolism in diabetes mellitus has acquired new significance with the acquisition of information concerning the important physiological role of insulin in the metabolism of fatty acids. Under normal conditions, the quantitative distribution of utilization of administered glucose is approximately as follows: oxidation to CO_2 and H_2O, 65 per cent; synthesis of fatty acids (lipogenesis), 30 per cent; glycogenesis, 5 per cent. In the presence of insulin deficiency there is actually greater impairment of lipogenesis from glucose than of oxidation of glucose. This fact, together with the existing stimulation of fat mobilization from adipose tissue, accounts for the striking loss of fat in severe diabetes, and contributes to the necessity for unduly large amounts of "acetate" to follow the pathways of ketogenesis and plasma cholesterol synthesis.

The capacity of extrahepatic tissues to utilize ketone bodies (oxidation to CO_2 and H_2O) is not impaired in diabetes. Consequently, in mild insulin deficiency, there may be neither excessive ketonuria (ketone bodies in urine) nor ketonemia (ketone bodies in blood plasma). With increasing deficiency, utilization fails to keep pace with formation, and ketonuria of increasing degree develops, followed by ketonemia. The latter is of more serious prognostic significance than the former, and can be detected by simple qualitative (semi-quantitative) tests. Ketonemia and, except in rare instances of severe renal failure, ketonuria, are invariably present in diabetic coma. These, together with hyperglycemia, glycosuria, and acidosis, enable this diagnosis to be made promptly and with ease.

Acidosis. (p. 335). Acidosis in diabetes is due in part to the existing ketosis and in part to the excessive loss of water with the associated elimination of excessively large quantities of base. Diabetic acidosis, therefore, is due to a primary alkali deficit which is most satisfactorily detected by one of the procedures designed to measure the alkali reserve of the body. Perhaps the most valuable of these from a clinical standpoint is the determination of the CO_2 content or combining power of the blood plasma which, in diabetes, may be reduced to a remarkable degree, the extent of reduction being more or less accurately indicative of the degree of acidosis. As a rule, coma is almost always present when the CO_2 content is less than 15 volumes per cent, but the individual threshold of consciousness varies within rather wide limits. Patients have been observed in profound diabetic coma with values of 28 volumes per cent while others with values of 10 to 12 volumes per cent have been merely drowsy or slightly stuporous. Although the plasma CO_2 more nearly parallels the clinical condition of patients with severe diabetes than does the blood sugar concentration, values below a certain critical level appear to have little significance with regard to prognosis. According to some, this critical level is about 25 volumes per cent. The presence of infection complicating diabetic coma is also important in determining the prognosis in any given instance. Since the introduction of insulin therapy, recovery from diabetic coma has been reported in patients with values for plasma CO_2 as low as 2 volumes per cent.

Electrolyte and Water Balance. Although the plasma chloride concentration is usually within normal limits in diabetes mellitus, hypochloremia is occasionally observed, particularly in advanced forms of the disease. Severe diabetes is accompanied by profound changes in electrolyte balance and in the electrolyte pattern of the blood. It has been found that the polyuria which attends the increasing glycosuria may be accompanied by an equally pronounced increase in the excretion of electrolytes, particularly sodium and chloride. With the progressive development of ketosis and acidosis, there is a secondary increase in the loss of water and electrolytes which continues until the condition is brought under control by proper therapeutic measures.

The following concurrent phenomena are believed to constitute the mechanism underlying the production of changes in the electrolyte pattern of the blood in diabetic acidosis. (1) Displacement of CO_2 from bicarbonate by ketone acids. (2) Although a portion of the ketone acids can be eliminated as free acids and a portion is neutralized by ammonia, a certain fraction is neutralized by fixed base, i.e., Na. The excretion of the latter fraction, in contradistinction to the former, withdraws base from the body which can be replaced only from extraneous sources. (3) Reduction of base is equivalent to reduction of the total electrolyte content of the body fluids and, consequently, dehydration, resulting from the simultaneous loss of body fluid. (4) The Na and Cl depletion is also contributed to by the polyuria incident to the glycosuria (p. 284), and also by vomiting (Cl), which occurs in many cases.

In diabetic coma, which is invariably accompanied by severe dehydration, the serum Na concentration is frequently decreased (120–130 mEq/liter), as is the Cl concentration, although usually not to the same degree (85–95 mEq/liter) unless vomiting is a prominent feature.

It should be noted that in the presence of dehydration the actual deficiency in body sodium and chloride is much greater than would appear to be indicated by the plasma chloride concentration and far exceeds the deficiency in bicarbonate. Because of the concomitant dehydration and hemoconcentration it is quite possible for the plasma chloride level to be actually elevated following a period of actual negative chloride balance and in the presence of marked chloride depletion. These observations have important therapeutic implications (p. 284).

The practical management of severe diabetic acidosis and coma has been improved considerably by better understanding of the incident disturbance in potassium metabolism (p. 290). During the period of progression of the diabetes, acidosis, and dehydration, fluid is lost in excess not only from the extracellular, but also from the intracellular compartment. Accordingly, the body is losing K (in the urine) as well as Na. In the presence of normal kidney function, increased urinary excretion usually suffices to prevent a significant rise in the concentration of K in the plasma, but, as the dehydration progresses, and the Na and Cl losses increase, diminishing renal function may become inadequate to remove promptly all of the K that enters the shrinking extracellular fluid compartment. Accordingly, the plasma K concentration may actually rise in the face of a serious K deficit.

Under such circumstances, active treatment with insulin (or insulin plus glucose) and solutions containing NaCl but no K, may result in an abrupt fall in the plasma K concentration (hypokalemia) to levels which are accompanied by serious and, at times, fatal, clinical manifestations (muscular weakness, respiratory difficulty, abdominal distention, cardiac irregularities, myocardial failure). This state of hypokalemia is due to: (*a*) restoration of extracellular fluid volume, thereby "unmasking" the K deficit; (*b*) passage of K from the extracellular fluid into the cells by virtue of the stimulation of glucose utilization by insulin (p. 32); (*c*) improvement of kidney function, which increases the loss of K from the extracellular fluid. These phenomena can be obviated by administration of potassium salts.

Negative Nitrogen Balance—Hypoproteinemia. Uncontrolled diabetes is accompanied by a state of negative nitrogen balance, abnormally large amounts of nitrogen being excreted in the urine. This is due to increased catabolism of protein in the absence of an adequate amount of insulin, due probably to an unopposed effect of certain anterior pituitary and adrenal cortical hormones. It has been found that these agents stimulate gluconeogenesis from protein in the liver; the growth hormone (anterior pituitary), which exhibits an anabolic effect (nitrogen retention) in the presence of normal amounts of insulin, causes increased nitrogen excretion (negative nitrogen balance) in diabetic animals. This phenomenon of excessive protein catabolism in the uncontrolled diabetic leads to a state of protein undernutrition with all of its deleterious consequences to the organism. In addition to a negative nitrogen balance, this may be evidenced by a decrease in the concentration of hemoglobin and plasma albumin.

Patients with diabetes mellitus are still encountered occasionally in whom injudicious dietary restriction has resulted in a state of extreme malnutrition, with diminution in the plasma protein concentration and, at times, edema. During periods of severe acidosis, particularly if accompanied by vomiting, the extent of reduction of the plasma protein concentration may be masked to a large extent by the dehydrating effects of vomiting and acidosis, with resulting hemoconcentration. If a patient in this condition were treated actively by the administration of adequate quantities of glucose, insulin, fluid and sodium chloride, the restoration of normal plasma volume would be accompanied by a more or less marked diminution in the concentration of plasma protein, which may be so low that edema develops. The development of this phenomenon is not uncommon following active treatment of patients in diabetic coma, with severe acidosis. The occurrence of albuminuria, hypoproteinemia, impairment of renal function and hypertension in patients with diabetes mellitus has been found to be associated with morphologic changes in the kidneys of a peculiar type, designated "intercapillary glomerulosclerosis" (Kimmelstiel-Wilson syndrome).

Nitrogen Retention. A moderate to marked increase in the nonprotein nitrogenous constituents of the blood may occur in patients with advanced diabetes mellitus, particularly in those in diabetic coma. In some instances this is due to the presence of complicating renal disease, such as intercapillary glomerulosclerosis, glomerulonephritis, advanced nephrosclerosis, or marked degenerative changes in the kidneys. In other instances, however, no significant renal lesions have been demonstrable at autopsy. The cause of nitrogen retention under such circumstances is not known, but it may be dependent upon the operation of one or more of several factors. Among these are: (1) dehydration, due to acidosis, diuresis, vomiting and limited fluid intake; (2) circulatory failure and extremely low arterial blood pressure (state of shock); (3) excessive catabolism of protein; (4) hyponatremia and hypochloremia; (5) renal functional impairment due to intercapillary glomerulosclerosis. No one of these factors has been shown to be operative in all cases manifesting this phenomenon.

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CHAPTER II

Lipid Metabolism

Although the total number of compounds that must be considered in discussing the metabolism of lipids does not equal, for example, the number of proteins and amino acids, the chemical types represented are much more heterogeneous. First are the fatty acid esters of glycerol (triglycerides, neutral fats) and of other alcohols (waxes). Then there are the "glycerophosphatides," some of which contain fatty acids (as esters), phosphoric acid, and a base (ethanolamine, choline, serine), and a few of which contain fatty aldehydes. The sphingomyelin type of phospholipid and the glycolipids contain the unusual basic alcohol, sphingosine, to which fatty acids are attached in amide linkage. The carbohydrate components of the glycolipids and the presence of inositol in certain phospholipids add to their diversity. Finally, substances must be mentioned that are related to the lipids, partly by metabolic connections, largely by common solubilities, e.g., the sterols and bile acids, other steroids, and the fat-soluble vitamins and provitamins.

In considering the metabolism of the individual constituents of the lipids, it is obvious that most of them are only remotely connected with the lipids. The carbohydrates of the glycolipids and the glycerol moiety of the fats and phospholipids are synthesized and degraded via pathways common to the general metabolism of carbohydrates. Serine, ethanolamine, and choline are more directly related to amino acids than to lipids in their anabolism and catabolism. In fact, the only constituents of lipids that have a completely "lipoid" metabolism are the fatty acids, most of the other components entering into this area of metabolism only at such points as they are incorporated into the lipid molecule in the process of its formation. Consequently, although the incorporation of all constituents into the lipids must be considered, discussion of anabolism and catabolism of the lipids consists largely in a treatment of the synthesis and degradation of the fatty acids themselves.

As a consequence of the diversity of compounds encompassed by the class of lipids, the treatment of the metabolism of these substances necessarily differs from that applicable to the carbohydrates and proteins. The individual carbohydrates are connected, more or less directly, with the glycolytic sequence of reactions, and a common thread of metabolism runs through the reactions of the proteins and even of the individual amino acids, with few exceptions. On the other hand, the metabolism of the individual types of lipids and their constituents must be considered independently, for practically no metabolic interrelations are known among these substances of a type characteristic of the other major foodstuffs.

Another unusual feature of lipid metabolism is the importance of certain phenomena of transport and deposition. Although mobilization of carbohydrate and protein from one site in the body to another certainly occurs, as does deposition of stores of surplus (at least in the case of carbohydrate), these phenomena do not occupy the prominent position or display such aberrations under abnormal conditions as in the case of lipids.

NATURE OF LIPIDS

Lipids are defined as naturally occurring substances consisting of the higher fatty acids, their naturally occurring compounds, and substances found naturally in chemical association with them. Probably the most important role of lipids, from the quantitative viewpoint, is that of fuel. In some respects lipid is even superior to carbohydrate as a raw material for combustion, since it yields more heat per gram when burned, and furthermore can be stored by the body in almost unlimited amount, in contrast to carbohydrate. Some deposits of lipid may exert an insulating effect in the body, while others may provide padding to protect the internal organs. The nervous system is particularly rich in lipids, especially certain types which do not occur normally in such high concentrations elsewhere in the organism, but unfortunately we know little about the functions of these particular substances in the nervous system or other tissues. Some compounds derived from the lipids are important building-blocks of biologically active materials; e.g., acetic acid (a normal breakdown product of the fatty acids, as will be seen later) can be used by the body to synthesize the rather complex compound, cholesterol, which in turn can give rise to certain hormones. One of the important functions of dietary lipid is that of supplying so-called "essential fatty acids," compounds which cannot be synthesized by the animal organism. Compounds of lipids with proteins, lipoproteins, may be important constituents of many natural membranes, such as cell walls, and are involved in a number of other phenomena which may prove to be of clinical significance.

Most of the lipid material occurring in nature differs from the carbohydrates and the proteins in being insoluble in water and soluble in certain organic solvents (ether, benzene, chloroform, etc.). The fat depots of animals contain mainly neutral fat, by which is meant esters of glycerol with three fatty acid molecules. In contrast, most cells other than adipose tissue contain much less fat; their lipids consist largely of phospholipids and cholesterol. The brain is particularly rich in cholesterol (largely in the free state), phospholipids, and glycolipids. The lipoproteins have been scarcely investigated as a group, but a possible clue to their future importance is given by current researches which have attempted to relate the occurrence of certain lipoproteins in the plasma to atherosclerosis.

Attention will be directed mainly to those classes of lipids that are involved in abnormalities of clinical significance.

Fats (Triglycerides). The storage lipid of animals and the chief lipid constituent of the diet is neutral fat (triglycerides). These compounds consist of three molecules of fatty acid esterified to a molecule of glycerol. They differ from one another in the nature of their constituent fatty acid, i.e., variety, chain length, saturation or unsaturation. Hydrolysis of the glycerides proceeds by the addition of the elements of water across the ester linkages, producing, successively, diglycerides (and one fatty acid), monoglycerides (and two fatty acids), and glycerol (and three fatty acids).

Glycerol is closely allied, chemically and metabolically, to carbohydrates, with which it is interconvertible. The fatty acids differ in chain length (molecular size) and with respect to the presence of double bonds between certain carbon atoms (unsaturation). The poly-unsaturated fatty acids, viz., linoleic (two double bonds), linolenic (three double bonds), and arachidonic (four double bonds), are termed "essential" fatty acids, since there is evidence that they cannot be synthesized in the body, are essential for normal nutrition, and must therefore be provided in the diet.

Neutral fat is present normally in the liver ($<$ 1-2 per cent) and, especially after a lipid-containing meal, in the blood plasma (p. 104). In extrahepatic tissues, it is confined normally largely to the fat depots (adipose tissue). Relatively large quantities of unsaturated fatty acids are found in the liver, as compared with other tissues.

The body uses fats mainly as fuel. On a weight basis, the calorific value of fat (9.3 Cal. per gram) is more than twice that of carbohydrate or protein (4.1 Cal. per gram), it can be stored in practically anhydrous condition and in almost unlimited amounts, and it is metabolically sufficiently labile to be readily mobilized from the fat depots when needed. Nevertheless, carbohydrate, and not fat, is the preferred fuel of the body, and any attempt to utilize appreciable quantities of fat without concomitant degradation of adequate amounts of carbohydrate can lead to serious consequences, e.g., ketosis, hypercholesterolemia, hyperlipemia, fatty liver (p. 106).

Phospholipids (Phosphatides). These are so designated because they contain phosphoric acid. The most important members of this group are (*a*) lecithins, (*b*) cephalins, and (*c*) sphingomyelins.

Phospholipids are the dominant type of lipid in tissues other than adipose. Lecithins and cephalins are found in practically all tissues. The concentration of sphingomyelins is generally low except in brain and lung tissue, where it is a prominent constituent of myelin sheaths. In most muscles, phospholipids consist almost entirely of lecithins and cephalins, the former usually predominating. Heart muscle contains more than skeletal and smooth muscles. In general, the phospholipid content varies with the level of habitual activity of the muscle; e.g., it is higher in muscles of active animals than in corresponding muscles of sedentary animals, even of the same species.

LECITHINS. A typical lecithin consists of glycerol, to which are esterified two fatty acids (frequently at least one is unsaturated) and phosphoric acid, which in turn is attached to the organic base, choline (trimethylethanolamine).

CEPHALINS. Cephalins differ from lecithins with respect to the base attached to the phosphoric acid. Two types are known. In one, the base is ethanolamine, in the other, serine, an amino acid. They, too, often contain one or two unsaturated fatty acids.

SPHINGOMYELINS. These contain, in addition to phosphoric acid and choline, a complex base called "sphingosine" and a fatty acid.

Early studies led to the view that there are two types of lipids, from the standpoint of metabolic lability: (1) a fraction undergoing wide quantitative fluctuations in tissues in response to dietary changes, the "*élément variable*"; (2) a fraction, predominantly phospholipid, characterized by constancy of concentration, even in severe inanition, the "*élément constant.*" However, modern studies with isotopes contradict this point of view, and render dubious any claim of durability of any constituent of the tissues.

Although the phospholipids can scarcely be considered metabolically inert, this fact in itself does not prevent these compounds from serving a structural function. Indeed, it is probable that phospholipids participate in the lipoprotein complexes which are believed to constitute the matrix of cell walls and membranes, and probably of such structures as mitochondria and microsomes. In this role, they impart certain physical characteristics to these structures that are of functional significance, viz., high permeability toward certain hydrophobic molecules and lysis by surface-active agents such as bile salts and detergents. The phospholipid: cholesterol ratio may be of significance (p. 112).

Certain enzymes appear to require tightly bound phospholipid for their action. Whether or not the phospholipid deserves the status of a coenzyme, the fact remains that its removal from the enzyme protein irreversibly inactivates the latter.

Substances having the properties of phospholipids (possibly cephalins) are believed to function as parts of both "thromboplastin" and "antithromboplastin" in the mechanism of blood coagulation. The specific compounds involved have not been identified.

It has been thought for some time that phospholipids act as carriers of fatty acids into and out of cells, particularly during absorption of fatty acids from the intestine. This view has undergone radical revision as a result of studies with isotopes, this purported function having been reduced to, at best, a minor status. The increased turnover rate of the phospholipids of the intestinal mucosa during fat absorption is inadequate to account for any major fraction of absorbed fatty acids. Moreover, the phospholipid fraction of lymph does not transport a significant proportion of absorbed fatty acids from the intestine to the thoracic duct.

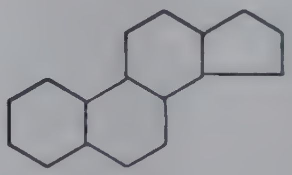
It has also been widely held that phospholipids serve as an important medium of transport of fatty acids from one tissue to another, e.g., between liver and depots. This concept has had to be virtually abandoned. According to present information, the phospholipids of the plasma are synthesized almost exclusively in the liver, and are removed from the plasma mainly by the same organ. Other tissues synthesize their own phospholipids, liberate practically none into the plasma, and absorb very little from that source. A possible indirect role of phospholipids in the blood is suggested by recent studies of the relation between the phospholipid:cholesterol ratio of plasma and the state of aggregation of lipoproteins (p. 112).

That phospholipids play some role in the metabolism of fatty acids, at least in the liver, seems probable from observations that the turnover of phospholipids is correlated with the intensity of fat metabolism (fat feeding and diabetes). However, at this time, the specific nature of the function served by these compounds is a mystery.

Glycolipids. The most important members of this group are the cerebrosides. These usually contain "sphingosine," a fatty acid, and galactose (galactolipids). Some contain glucose instead of galactose.

Cerebrosides are present in many tissues, but normally in low concentration, except for nervous tissue. Large amounts are found in the white matter of brain and in the myelin sheath of nerves.

Steroids. This designation is applied to a large group of compounds having in common a structure based on the cyclopentanophenanthrene nucleus. They have greatly diversified physiological properties and include such substances as: (*a*) provitamins D; (*b*) cholesterol; (*c*) bile acids; (*d*) important hormones, viz., adrenocortical, androgens, estrogens, progesterone; and (*e*) certain potent carcinogens. Certain of the steroids occur in nature in association with lipids, either by reason of mutual solubility, or owing to some actual metabolic relationship.



Cholesterol is a steroid alcohol and, in tissues (including blood plasma) outside of the nervous system, exists partly in the form of esters with fatty acids, although, with the exception of the liver, plasma, and adrenal cortex, it is present largely in the free state. The amount of cholesterol in muscles is apparently related to the degree of automatic activity; e.g., in man, smooth muscle invariably contains more than skeletal muscle, heart muscle occupying an intermediate position. Inasmuch as their phospholipid contents are approximately the same, the phospholipid: cholesterol ratio is lower in smooth (5:1) than in skeletal (10–16:1) muscle. The significance of these relationships is not known.

It is well established that cholesterol is the parent substance of the cholic acid component of the bile acids, this transformation occurring in the liver. It is also convertible into progesterone, testosterone, and the adrenocortical hormones. However, in the latter two instances, there is some question as to whether it is a direct, obligatory precursor of these steroid hormones. Despite the fact that administration of ACTH results in simultaneous increase in adrenocortical hormone formation and decrease in adrenal cholesterol content, there is evidence that both cholesterol and these steroid hormones are probably formed from a common precursor, e.g., acetate.

Cholesterol is also related, chemically and biologically, to vitamin D. In the liver and intestinal wall (and possibly in the skin), cholesterol undergoes dehydrogenation to 7-dehydrocholesterol, which is transformed to vitamin D under the influence of ultraviolet light.

Lipoproteins. It is probable that many of the substances described in this chapter do not exist as such in their natural state, and represent what the purists would call artifacts of the laboratory. This is a reflection of the primitive state of intracellular biochemistry (and unfortunately the situation is not confined to the lipids). From the available evidence, it appears that many of the lipids are combined with proteins in the tissues. These combinations are called "lipoproteins." The nature of the linkage between the lipid and protein moieties is not known; it is usually stable toward nonpolar solvents, such as ether, but can frequently be disrupted by more highly polar substances, such as alcohol. Despite the evidence for the widespread occurrence of lipoproteins, the technical difficulties in handling these materials have been such that, until the very recent introduction of newer physicochemical methods, few members of this group were characterized as chemical entities.

It is widely assumed that the cell membrane is a lipoprotein structure of some type, and, although there is not much direct evidence available, the general properties of cell membranes are at least consistent with this view. Certain of the internal structures of the cell also contain lipids, apparently in combination with proteins. This is the case with visible entities, such as the nucleus and mitochondria, as well as the submicroscopic particles (microsomes).

The thromboplastic protein isolated from lung (p. 83) has been characterized as a lipoprotein. The lipid portion, as in the case of many lipoproteins, consists of more than one type of lipid. Egg yolk contains a lipoprotein known as lipovitellin, the lipid fraction consisting largely of phospholipids. A number of bacteria have been shown to contain firmly bound lipids, although it is not certain in some cases whether the lipids are bound to protein or to polysaccharides. Certain animal viruses appear to contain firmly bound lipids also, but this is not true of the viruses thus far isolated from plant sources. The film which stabilizes the fat droplets in milk appears to be a combination of protein and phospholipid. Most, if not all, of the lipid in blood serum is carried in the form of lipoprotein complexes. The statement also holds for the lipid-soluble vitamins, the carotenoids, and the steroids. Since the presence of lipid causes the lipoprotein molecule to have a lower density than that of ordinary proteins, it is possible, by proper adjustment of the experimental conditions, to force lipoproteins to travel toward the axis instead of toward the periphery of an ultracentrifuge.

The flotation (negative sedimentation) determinations are made in a medium of known density at a given temperature. The rate of upward migration of a molecule under these conditions is expressed as "Svedberg flotation units" (S_f), one unit being equal to 10^{-13} cm./second/dyne/gram at 26° C. in a medium of NaCl solution, sp.gr. 1.063. Molecules exhibiting a flotation rate of 10 S_f units are referred to as S_f 10 molecules. Blood plasma has been shown to contain molecules of the classes S_f 2 to S_f 40,000 in various physiological and pathological states, the size of the lipoprotein molecule generally increasing with increase in the S_f number. The higher numbered classes contain particles that fall into the category known as "chylomicrons."

DIGESTION AND ABSORPTION

Fats are triglycerides of fatty acids, consisting of the combination of three fatty acid radicles with one molecule of glycerol. Palmitic, stearic and oleic acids, the most important fatty acids in the animal organism, are straight-chain acids with an even number of carbon atoms, the first two being fully saturated and the last unsaturated.

Linoleic, linolenic and arachidonic acids, all unsaturated, have been termed essential fatty acids, since their absence from the diet of rats results in disturbances of growth, reproduction and lactation, and in hematuria, scaly skin, and caudal necrosis. Strictly speaking, only linoleic acid is essential, since it will prevent the development of these deficiency manifestations. There is only indirect and incomplete evidence that this fatty acid is essential for humans, which suggests, however, that it may be necessary for maintaining the integrity of the skin.

Fat-splitting enzymes (lipases) are present in the secretions of the stomach, pancreas and small intestine. The most important of these is pancreatic lipase. Under conditions of normal gastric secretion, gastric lipase possesses little or no functional activity, but some degree of fat digestion may occur in the stomach if the acidity of the gastric juice is significantly decreased. Under the influence of these enzymes, fats are broken down into fatty acids and glycerol if the reaction is allowed to proceed under optimum conditions for a sufficiently long time. It has been pointed out that under conditions operative in the intestine during the period of active fat digestion and absorption, little if any free glycerol is formed, the products of lipase action being fatty acid and mono- and diglycerides.

The digestion of fat is enhanced to some extent by the presence of bile salts. These substances exert their influence in this connection in the following manner: (1) They serve as activators of pancreatic lipase. (2) They aid directly in the emulsification of ingested fat. Due to their property of lowering surface tension, in the presence of bile salts fat globules are reduced in size, and the total surface area exposed to the lipolytic enzyme is thereby increased, digestion being correspondingly facilitated. (3) They aid in dissolving fatty acids which, in solution, also lower the surface tension and favor finer emulsification. In combination with alkali, fatty acids form soaps, which also aid in the emulsification process.

The physiological significance of bile salts and soaps in this connection has been questioned on the basis that bile salts produce only a crude and relatively unstable emulsion and that soaps, although excellent emulsifying agents in alkaline solution, will not cause fine emulsification at a pH below 7.5. This is considerably higher than the pH usually present in the upper intestine.

There is considerable difference of opinion as to both the completeness of hydrolysis of fats in the bowel under physiological conditions and the mechanism of absorption of the digestion products. Absorption occurs most actively in the upper small intestine.

The two main current viewpoints on this subject are embodied in the so-called (1) "lipolytic" and (2) "partition" hypotheses. According to the lipolytic hypothesis, fat in the intestine undergoes emulsification (by bile acids, etc.,

p. 556) and more or less complete hydrolysis (by lipase) to fatty acids and glycerol, which are then absorbed. The fatty acids tend to form soluble complexes with bile acids (hydrotropic action), facilitating their entrance into the epithelial cells of the intestinal mucosa. Within these cells, the fatty acids are separated from the bile acids and are reincorporated into triglyceride (neutral fat) and perhaps also phospholipid molecules, which are passed largely, by way of the lacteals, via the intestinal lymphatics to the thoracic duct and thence into the systemic circulation. The bile acids return to the liver mainly by way of the portal circulation (enterohepatic circulation of bile acids). Glycerol may be absorbed into the portal blood, as may also perhaps a small percentage of the fatty acids (particularly short-chain).

According to the partition hypothesis, fat in the intestine undergoes emulsification and only partial hydrolysis to glycerol, fatty acids, mono- and diglycerides. The fats are thus "partitioned" into fatty acid and glyceride (mono-, di-, and tri-) fractions. The fatty acids are absorbed as such (with the aid of bile acids) and pass mainly to the liver by way of the portal vein; glycerides of short-chain fatty acids also may take this route. The glycerides, with particle diameters below 0.5 μ as a result of emulsification, are absorbed as such by the intestinal epithelial cells and pass into the lymph stream (intestinal lymphatics and thoracic duct) and thence into the systemic circulation.

Experimental findings contradict certain features of both of these hypotheses. Inasmuch as no entirely acceptable mechanism can be presented on the basis of available evidence, it seems advisable merely to indicate certain facts that are apparently fairly well established.

The conditions existing normally in the small intestine are not optimal for pancreatic lipase activity. The products of fat digestion presented for absorption (small intestine) probably include unchanged fats (triglycerides, di- and monoglycerides), fatty acids, and glycerol.

After ingestion of large amounts of either neutral fats or free fatty acids (particularly long-chain), the lipid content of the intestinal and thoracic duct lymph increases enormously. This increase is largely in neutral fat in both cases. There is little if any free fatty acid in either blood or lymph. Phospholipid increases also (lymph), but to a relatively slight extent. Ingestion of either neutral fats or fatty acids results in an equal degree of lipemia (increased plasma lipid content, mainly neutral fat). These observations suggest that triglycerides and phospholipids are synthesized from fatty acids during the process of absorption (intestinal epithelial cells).

Simultaneous administration of choline accelerates absorption of fats. The mechanism of production of this effect is not known. Choline increases the turnover of phospholipids in the intestine. However, the formerly prevailing view that phosphatides are important intermediates in the transport of fat across the intestinal mucosa has been refuted. It has been found that there is no increase in the amount or turnover rate of phosphatides in the intestinal wall during fat absorption.

When labeled, free, long-chain fatty acids are fed, 70 to 90 per cent are recovered in the thoracic duct lymph. This is evidence against the partition hypothesis, according to which free fatty acids are absorbed mainly into the portal circulation and glycerides mainly by the lymphatics. However, short-chain fatty acids (e.g., butyric) may be preferentially absorbed by the portal route.

Despite certain contradictory evidence, absorption of fatty acids is apparently facilitated by bile acids (hydrotropic action). Fatty acids may also combine with cholesterol in the intestinal mucosa, and enter the lymph as cholesterol esters.

Phosphatides, entering the intestine in the food or in digestive secretions (e.g., bile, intestinal juice), may be absorbed as such, perhaps more readily than triglycerides because of their hydrophilic nature. Lecithins and cephalins may also be hydrolyzed in the intestine by lecithinases; lecithinase A apparently removes the unsaturated, and lecithinase B, the saturated fatty acid. The end-products of complete digestion by these enzymes are free fatty acids, and glycerylethanol-amine in the case of lecithins and glycerylphosphorylethanolamine or -serine in the case of cephalins. The glycerylphosphate linkage may be hydrolyzed, probably in the intestinal mucosa, by phosphomonoesterases.

Cholesterol enters the small intestine in the food (mainly cholesterol esters) and in the intestinal secretions and bile (mainly free cholesterol). Pancreatic juice, bile, and intestinal epithelial cells contain (1) cholesterol esterase, which hydrolyzes cholesterol esters to free cholesterol and fatty acids, and (2) other enzymes which esterify cholesterol. Esterification apparently occurs actively during the process of absorption; after feeding free cholesterol, most of the increased cholesterol in the thoracic duct lymph is in the ester form. The passage of cholesterol from the intestinal lumen into the mucosal epithelium may be facilitated by the formation of complexes with bile acids (hydrotropic action).

METABOLISM OF FAT

Immediate Fate of Dietary Lipid. Newly absorbed lipid reaches the plasma mainly by way of the lymphatics (thoracic duct to subclavian vein) (p. 87), manifesting itself largely in the neutral fat fraction of the plasma lipids, although increases occur also in other lipids during the absorptive period (p. 104). Neutral fat is taken up rapidly by fat cells (adipose tissue) and, secondarily, by the liver, the proportion going to each of these sites being determined by the physiological state of the organism (p. 91). The comparatively small dietary contributions to other plasma lipids are probably removed chiefly by the liver, since they are taken up by extrahepatic tissues relatively slowly.

Anabolism and Catabolism of Fatty Acids. The fatty acid moieties of the lipids of a given tissue are derived from (1) the diet, (2) transfer from other tissues, or (3) synthesis *in situ* from carbohydrate or protein. Although there is definite evidence of extrahepatic synthesis of fatty acids (even in the fat depots, which were formerly considered rather inert metabolically), it is probable that the liver is the major site of this phenomenon. Net formation of fatty acids occurs as a result of an intake of carbohydrate in excess of immediate requirements, but it has been demonstrated by the use of isotopes that a "steady-state" conversion proceeds continually, the catabolic reactions keeping pace with the anabolic.

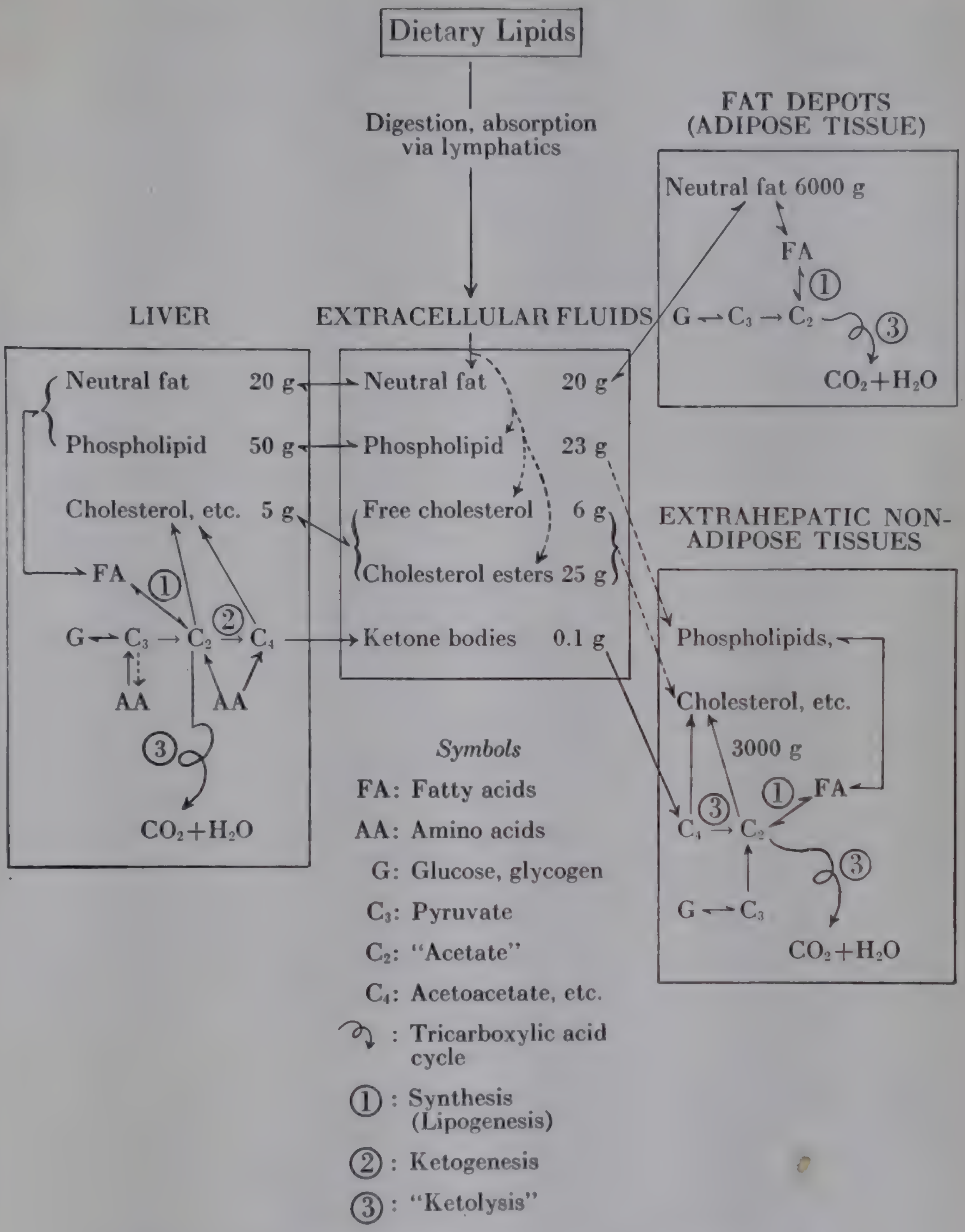

Dietary Lipids
Digestion, absorption via lymphatics
FAT DEPOTS (ADIPOSE TISSUE)
Neutral fat 6000 g
FA
①
G → C_3 → C_2
③
CO_2+H_2O
LIVER
EXTRACELLULAR FLUIDS
Neutral fat 20 g
Phospholipid 50 g
Cholesterol, etc. 5 g
FA
①
G → C_3 → C_2 → C_4
②
AA
AA
③
CO_2+H_2O
Neutral fat 20 g
Phospholipid 23 g
Free cholesterol 6 g
Cholesterol esters 25 g
Ketone bodies 0.1 g
EXTRAHEPATIC NON-ADIPOSE TISSUES
Phospholipids,
Cholesterol, etc.
3000 g
FA
①
③
C_4 → C_2
G → C_3
③
CO_2+H_2O
Symbols
FA: Fatty acids
AA: Amino acids
G: Glucose, glycogen
C_3: Pyruvate
C_2: "Acetate"
C_4: Acetoacetate, etc.
: Tricarboxylic acid cycle
① : Synthesis (Lipogenesis)
② : Ketogenesis
③ : "Ketolysis"

Fig. 13. Over-all metabolism and distribution of lipid in 70 kg. man.

Limitations to the synthesis of fatty acids are indicated by the existence of certain highly unsaturated fatty acids which are essential dietary factors.

Carbohydrate is the major raw material for the synthesis of fatty acids. Pyruvic acid forms "active acetate" (acetyl-CoA), which can take a variety of metabolic paths, one of which is fatty acid formation (p. 13). This is accomplished presumably by successive condensation, reduction, and dehydration processes, essentially the reverse of the reactions involved in the oxidative degradation of fatty acids to "acetate." There are also metabolic pathways available for the synthesis of fatty acids from amino acids. The glucogenic amino acids (p. 136) are convertible to pyruvic acid; the ketogenic amino acids (leucine, tyrosine, phenylalanine) form acetate or acetoacetate directly, both of which are lipogenic.

Insulin appears to be essential for the formation of long-chain fatty acids from acetate, i.e., for lipogenesis from carbohydrate. Under normal conditions, in a well-nourished animal (rat), about 30 per cent of administered glucose takes this metabolic path. In the absence of insulin, this process of lipogenesis from carbohydrate suffers quantitatively more than does the oxidation of glucose to CO_2 and H_2O. This indicates an important direct disturbance of fat metabolism in diabetes mellitus, in addition to the indirect consequences of impaired utilization of carbohydrate.

Fatty acids are catabolized primarily by oxidation, the extent and rate varying with the physiological state of the organism. Fatty acids are degraded to 2-carbon units, formerly called "active acetate," now identified as acetyl-CoA. Any such fragments formed from fatty acids in the extrahepatic tissues are immediately channeled into the tricarboxylic acid cycle (p. 8) for oxidation. Owing to circumstances which are not fully understood, but which may have something to do with the availability of oxalacetate, the facilities for entry of acetyl-CoA into the cycle in liver are rather easily overloaded. When the rate of formation of acetyl-CoA outstrips the capacity of the hepatic tricarboxylic acid cycle, condensation occurs between pairs of acetyl groups, forming acetoacetic acid ("ketogenesis"). In a side reaction, a significant proportion of this compound may be reduced to β-hydroxybutyric acid. Liver differs from other tissues also in its inability to split acetoacetate once it is formed. Acetoacetic acid, acetone (formed by the spontaneous decarboxylation of acetoacetic acid), and β-hydroxybutyric acid (collectively called "ketone bodies") therefore are sent into the blood stream under normal circumstances ("ketonemia"). Excessive ketonemia is designated "ketosis" (p. 94). The extrahepatic tissues have a great capacity for cleavage and oxidation of ketone bodies ("ketolysis"), which, however, may be exceeded, resulting in ketosis and excretion of detectable amounts of ketone bodies in the urine ("ketonuria").

Anabolism and Catabolism of Lipids. Within each tissue, fatty acids and other constituents of lipids are involved in a constant interchange between free and bound molecules. The rate of "turnover" of each type of lipid, as well as the general direction of net movement (synthesis or degradation), is determined by the chemical character of the lipid, its location in the body in general and in the tissue in particular, as well as by the physiological exigencies of the moment.

Lipid Mobilization. Lipids synthesized or stored in one tissue may, in response to physiological demands, be transferred to another site. The mechanism of transport in the blood plasma appears to involve lipoproteins (p. 101). The level of lipid in the blood is a resultant, on the one hand, of the rate of influx from the diet, synthesis in such tissues as the liver, and mobilization from the fat stores of the depots, and on the other, the rate of oxidation in the various tissues and deposition in the depots. Excessive levels of blood lipid ("hyperlipemia") may result from an imbalance between these sets of factors.

Endocrine influences play an important role in the mobilization of fat from adipose tissue stores. Increased secretion of anterior pituitary growth hormone apparently constitutes the "trigger mechanism" for this mobilization, but increased secretion of 11-oxygenated adrenocortical hormones is also essential. Under the influence of these factors, increased amounts of fat enter the plasma from the depots, and are carried to the liver, where they undergo a variety of metabolic changes, the most important of which, perhaps, is the oxidative degradation of their constituent fatty acids to "acetate" (p. 90).

In periods of nutritional plenty, dietary lipids, as well as those synthesized from carbohydrate in the liver, are sent to the depots for storage. In "lean" times, these stored materials are mobilized from the depots to the liver (and possibly other tissues) for oxidation. The concentration of lipid in the liver, then, is a resultant of the rates and directions of mobilization of lipid between liver and depots, as well as of the rates of synthesis and utilization within the liver. Imbalances may result in the development of "fatty liver" (p. 106). Agents which promote the clearance of lipid from the liver are said to be "lipotropic."

ENDOCRINE INFLUENCES IN LIPID METABOLISM

Hormones play an important role in the coordination of various phases of lipid metabolism and in their integration with carbohydrate metabolism (p. 93). The most important hormones in this connection are insulin, the 11-oxygenated adrenal cortical hormones, and anterior pituitary factors (growth hormone, or some principle intimately associated with it; adrenocorticotrophic hormone, acting via the adrenal cortex).

Insulin. Administration of insulin is followed by increased synthesis of fatty acids from 2-C fragments (lipogenesis), representing one phase of the increased utilization of glucose induced by this agent. Ketogenesis is depressed, another consequence of the stimulation of glucose utilization in the liver, which increases the supply of oxalacetate for incorporation of 2-C fragments from fatty acid into the Krebs cycle (p. 8).

As in the case of carbohydrate metabolism, there is some degree of functional antagonism between insulin and the lipid-active adrenocortical and anterior pituitary hormones at certain points. In the absence of adequate amounts of insulin, e.g., in pancreatic diabetes, decreased lipogenesis and increased ketogenesis, referable to the insulin actions indicated above, are accompanied by acceleration of mobilization of fat from the fat depots, with consequent increase in blood and liver lipids (p. 75). These phenomena are attributable to increased amounts or activity of the adrenal and pituitary hormones (q.v.), or to increased sensitivity of the depot fat to their action, unbalanced by normal amounts of insulin. Insulin deficiency is accompanied also by increased synthesis of cholesterol (liver) from 2-C fragments from the increased amounts of fatty acid mobilized from the fat stores; the plasma cholesterol rises.

Adrenocortical Hormones. Administration of adrenal oxysteroids (p. 433) is followed by diminished lipogenesis from carbohydrate and increased ketogenesis, these hormones antagonizing the actions of insulin in these respects.

Acting in conjunction with a fat-mobilizing principle of the anterior pituitary, apparently growth hormone or some factor intimately associated with it, these hormones stimulate mobilization of fat from the fat depots, with consequent increase in plasma and liver lipids (p. 106).

Anterior Pituitary Hormones. Administration of anterior pituitary extracts is followed by increased mobilization of fat from the fat depots, with loss of body fat and increase in plasma and liver lipids and in ketogenesis. The fat mobilizing effect is attributable primarily to the growth hormone ("trigger action") or some factor intimately associated with it, but requires also an increase in adrenal oxycorticoids. Neither factor alone can produce this effect.

Thyroid Hormone. Administration of thyroxine is followed by a decrease in plasma cholesterol and phospholipids, which increase following removal of the thyroid gland. The mechanism of action of thyroxine in this connection is not known.

METABOLIC INTERRELATIONS OF LIPIDS, CARBOHYDRATES, AND PROTEINS

Figure 14 illustrates the metabolic interrelations between the major types of lipids and other foodstuffs.

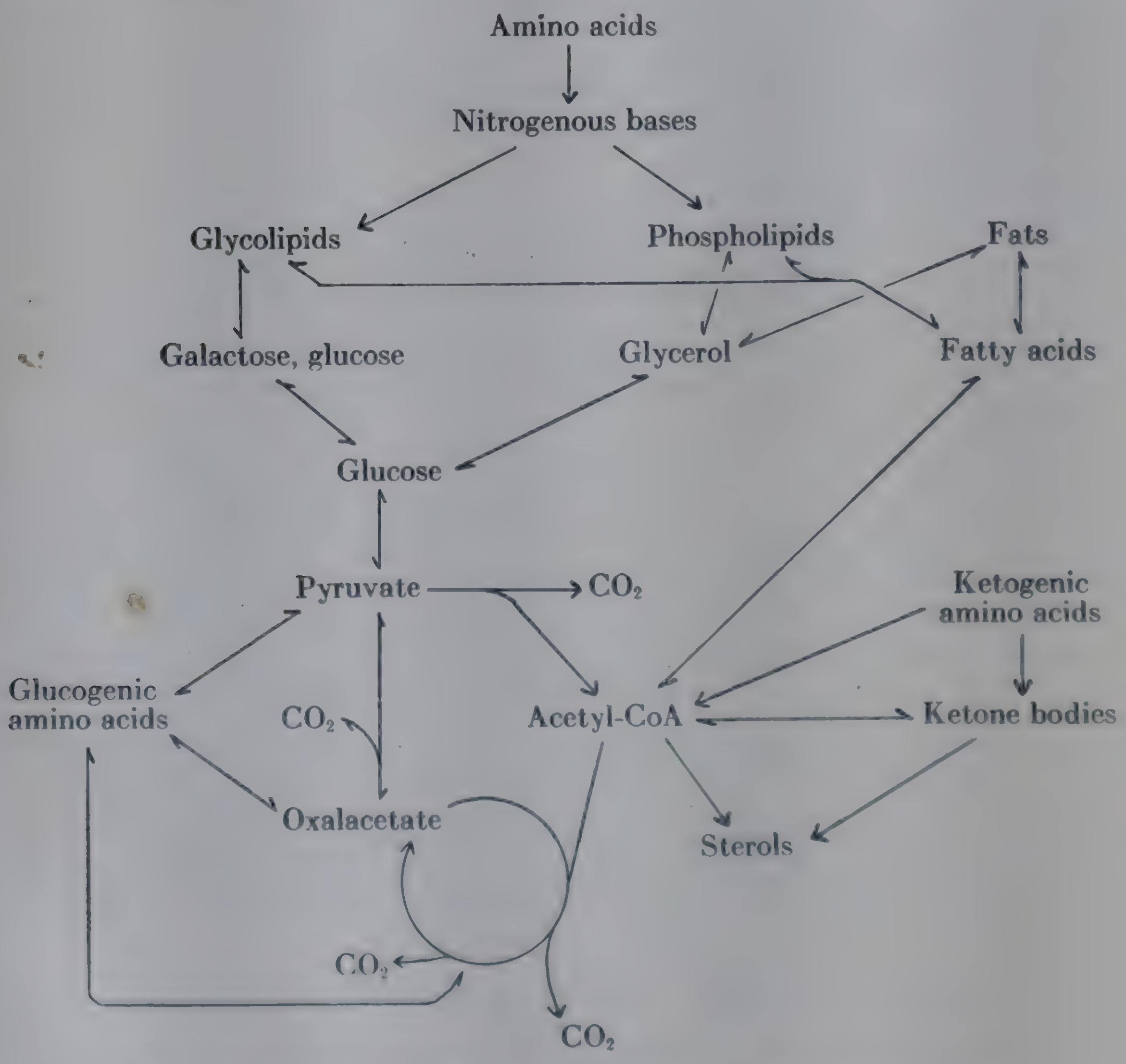

Amino acids
Nitrogenous bases
Glycolipids
Phospholipids
Fats
Galactose, glucose
Glycerol
Fatty acids
Glucose
Pyruvate
CO_2
Ketogenic amino acids
Glucogenic amino acids
CO_2
Acetyl-CoA
Ketone bodies
Oxalacetate
Sterols
CO_2
CO_2

Fig. 14. Interrelation of lipid, carbohydrate, and protein metabolism.

The lipids do not contribute significant quantities of material to the synthesis of amino acids. Glycerol is the only major raw material which may be provided for the synthesis of carbohydrate from lipid sources, although it is probable that the usual net flow of this substance is in the reverse direction. The characteristic relation of lipids to other foodstuffs involves either synthesis of the former from the latter, or some type of coupling between the rates of synthesis and degradation of the two families of compounds.

Carbohydrate is converted to lipid in various ways. Small amounts of galactose (and in some cases glucose) are undoubtedly provided for the synthesis of cerebrosides and gangliosides. The glycerol moiety of the triglycerides and glycerophosphatides is formed readily from the intermediates of glycolysis. However, the major conversion in this direction, and the one usually meant by the phrase, "synthesis of fat from carbohydrate," is the formation of fatty acids from acetyl units, which are in turn derived from the oxidative decarboxylation of pyruvic acid. The irreversibility of this decarboxylation, incidentally, forms the basis of the current view that fatty acids cannot be converted into carbohydrate.

The concluding statement of the previous paragraph seems to be belied by many experiments with isotopes, in which labeled carbon atoms from fatty acids have been shown to be incorporated into glucose or glycogen. The contradiction is more apparent than real, however. Inspection of the reactions of the tricarboxylic acid cycle (p. 8) subsequent to the condensation of acetyl-CoA and oxalacetic acid reveals that the two carbon atoms lost as CO_2 from the cycle are not the same atoms which entered the cycle as acetate. The carbon atoms of the acetate finally appear in the regenerated oxalacetic acid, which can form pyruvic acid by decarboxylation. Hence a labeled atom from acetate or a fatty acid can be incorporated into glucose or glycogen. Nevertheless, the "material balance" shows that two carbon atoms have reacted with a molecule of oxalacetic acid to form two molecules of CO_2 and regenerate the oxalacetic acid. Since no additional molecules of oxalacetic acid are formed, no net synthesis of pyruvic acid or carbohydrate from fatty acids has occurred, despite the transfer of carbon atoms from one to the other.

Amino acids contribute to the synthesis of lipids. Ethanolamine, choline, and serine, for example, components of the phospholipids, are derived from the pathways of protein metabolism. The ketogenic amino acids form acetic or acetoacetic acids, from which fatty acids can be derived. The glucogenic amino acids, directly or indirectly, form pyruvic acid, which can be used for the synthesis of either carbohydrate or fatty acids.

An intimate quantitative relationship exists between the metabolism of fatty acids and of carbohydrate. When the rate of oxidation of carbohydrate, the preferred fuel of the body, falters, fat is mobilized from the depots to the liver, probably by a mechanism involving the pituitary and adrenals (p. 91). The rate of degradation of fatty acids in the liver is then increased. The mechanism of the "coupling" involved is not known, but it seems reasonable to postulate that, when carbohydrate is oxidized at a rapid rate, the production of acetyl-CoA molecules from pyruvic acid results in virtual coverage of the condensation enzyme of the tricarboxylic acid cycle, thus forcing the equilibrium of fatty acid $\rightleftharpoons$ acetyl-CoA toward synthesis. Adequate energy for the anabolic pathway toward fatty acids is also provided by the oxidation of pyruvic acid. There are several reasons, therefore, why a diminution in the number of pyruvic acid molecules being oxidized per unit time will automatically cause an augmented breakdown of fatty acids. This metabolic stratagem, however, carries with it dangers of its own. A decreased rate of oxidation of carbohydrate results, in the liver at least, in a shortage of oxalacetic acid, due to various wasteful side reactions. The resulting increase in ketogenesis and its consequences are described elsewhere (below). It would appear, then, from the foregoing remarks that, whereas carbohydrate may be considered a competing substrate to fatty acids in one sense, it is a necessary partner in their oxidation in another.

Although this discussion of the metabolism of fatty acids and carbohydrates has stressed the net flow of metabolites in one direction or another, it may be well to conclude with the reminder that, regardless of net synthesis or catabolism or steady states, all groups of molecules connected metabolically interchange material continually, as indicated by isotopic labeling. Whether the physiological situation of the moment calls for oxidation predominantly of carbohydrate or of fat, a certain amount of conversion of carbohydrate to fat always takes place. These and other aspects of the "dynamic state of the body constituents" are considered in greater detail in connection with the metabolism of protein (p. 126).

KETOSIS

Ketosis is the term applied to the accumulation of excessive quantities of "ketone bodies," i.e., acetoacetic acid, β-hydroxybutyric acid, and acetone, in the body. Ketonuria refers to their excretion in excess in the urine. As indicated elsewhere (p. 90), these substances are formed in the liver, normally in relatively small amounts, and pass in the systemic circulation (0.2 to 0.8 mg./100 cc.) to the extrahepatic tissues, where they are oxidized to CO_2 and H_2O. On an average mixed diet up to 125 mg., expressed as β-hydroxybutyric acid, may be excreted in the urine of normal subjects daily, if sufficient carbohydrate is ingested.

The extrahepatic tissues have a great capacity for utilizing these ketone bodies (ketolysis). Acetoacetic acid is apparently "activated" and split into two molecules of acetyl-CoA ("active acetate"), which then enters the tricarboxylic acid cycle and is oxidized to CO_2 and H_2O. The liver, which is the site of formation of ketone bodies, apparently cannot utilize these substances.

Even under conditions of severe carbohydrate restriction or impaired carbohydrate utilization (e.g., diabetes mellitus), with marked ketosis, the utilization of ketone bodies in the extrahepatic tissues proceeds essentially normally. Consequently, the invariable cause of ketosis is excessive ketogenesis, not inadequate ketolysis, and the liver must be the organ involved in this phenomenon. As indicated elsewhere (p. 8), a molecule of oxalacetate is required for the entrance of each molecule of acetate into the tricarboxylic acid cycle, in which the acetate is oxidized to CO_2 and H_2O and the oxalacetate is regenerated. In the liver, but not elsewhere, the latter critical substance readily undergoes decarboxylation to pyruvate and, therefore, a continual supply of the latter must be provided, mainly from carbohydrate, in order to maintain an adequate concentration of oxalacetate. If this situation does not exist, as in insulin deficiency or carbohydrate deprivation, the acetate fragments (from pyruvate, fatty acids, and certain amino acids) are not adequately incorporated into the tricarboxylic acid cycle, and are diverted in excess into ketogenesis. Moreover, under such conditions, also, fat mobilization is stimulated (p. 91), and increased amounts of acetate are provided, for ketogenesis, from this source (i.e., fatty acids (p. 90).

When the capacity of the tissues for utilizing the ketone bodies is exceeded, the excess is excreted in the urine (ketonuria). When the capacity of the kidneys for excreting these substances is exceeded, they accumulate in the plasma (hyperketonemia or ketosis). Severe ketosis is accompanied by acidosis, bicarbonate being displaced by the acid anions, acetoacetate and β-hydroxybutyrate (primary bicarbonate deficit, p. 336). The fact should be emphasized, however, that there is no obligatory causal relationship between acidosis and ketosis. Ketosis does not occur in conditions accompanied by acidosis unless there is an associated state of carbohydrate deprivation (e.g., vomiting) or inadequate utilization. In fact, ketonuria and ketosis occur commonly in alkalosis due to persistent vomiting, unless glucose is administered parenterally.

Ketonuria and ketosis may occur in a variety of clinical conditions which have as a common denominator inadequate supply or utilization of carbohydrate:

(1) Diabetes mellitus, increasing with increasing severity of the condition (p. 74).

(2) Carbohydrate starvation, as seen in vomiting, due to any cause, severe anorexia and starvation due to other causes, and postoperatively, if glucose is not given parenterally.

(3) At times in protracted hyperinsulinism, with depletion of liver glycogen, due to hypoglycemia, and consequent unavailability of carbohydrate in adequate amounts.

(4) The hepatic form of glycogen storage disease (p. 57).

(5) Acute increase in metabolism, with increased requirement for carbohydrate, as in thyrotoxicosis and fever.

(6) Ketonuria may occur in the Fanconi syndrome, as a reflection of both decreased renal tubular reabsorption of these organic acids and depletion of hepatic glycogen reserves incident to the loss of glucose in the urine in this condition (p. 62). Ketonuria has also been observed, although rarely, in other types of renal glycosuria. It may occur in severe form in phlorizinized animals (p. 61).

(7) Ketonuria can be induced by administration of growth hormone and 11-oxygenated adrenocortical hormones and occurs, occasionally, in the corresponding clinical conditions, i.e., acromegaly and Cushing's syndrome (decreased utilization of carbohydrate and increased fat mobilization) (p. 91).

FAT IN FECES

Fat is normally present in the feces in three forms, soap fat (combined fatty acids), free fatty acids, and neutral fat, the relative proportion of each being dependent somewhat upon the efficiency of fat digestion and absorption. There a e two methods commonly employed for the quantitative analysis of fecal fat, the dry method of Cammidge and the wet methods of Saxon and of Fowweather. The most popular clinical method is that advocated by Cammidge, which has been attacked as unsound by Fowweather who bases his opinion upon the following observations: "Any method in which the stools are dried by heat before analysis is open to serious objections. Stools are rarely neutral in reaction; many are alkaline. The heating of neutral fat in a finely divided state in the presence of water and particularly if the reaction of the material is alkaline, would be expected by any chemist to result in hydrolysis or splitting of the fat. Hence, the neutral fat content of feces after drying is not always the same as before drying. This is not merely a theoretical objection but has been shown to occur in practice."

Normal values, based upon the examination of dried feces, are as follows:

	Per cent of dry weight
Total fat	15–25
Neutral fat	10–15
Free fatty acids	9–13
Combined fatty acids (soaps)	10–15

Normal values, employing the wet method, are as follows:

	Per cent of total dry matter
Total fat	17.5
Neutral fat	7.3
Free fatty acids	5.6
Combined fatty acids (soaps)	4.6

Fowweather lays down the following criteria for abnormal fat excretion in adults and children over two years of age:

(*a*) Any specimen in which the total fat exceeds 25 per cent of the total dry matter of the feces is probably abnormal.

(*b*) Deficient fat digestion is suggested in any specimen in which the neutral fat exceeds 11 per cent of the total dry matter or 55 per cent of the total fat.

(*c*) Deficient fat absorption is suggested in any specimen in which the total fatty acid (free fatty acid plus soap) exceeds 16 per cent of the total dry matter and 75 per cent of the total fat.

Obviously, the fecal fat may consist of either unabsorbed fat, fat that has been excreted into the bowel, or both. Evidence is at hand which suggests that under normal and many abnormal conditions the fat of the feces is probably largely an endogenous excretion product. The amount excreted is quite constant under normal conditions and is independent of the diet, often remaining unchanged during periods of starvation. In the past, considerable clinical significance was attached to studies of the amount and partition of fecal fat in relation to disturbances of fat absorption and digestion. In cases of severe diarrhea, the fat content of the feces may be unusually high, due to the increased rapidity of passage of food through the bowel, with consequent impairment of absorption. This occurs also in patients with gastrocolic fistula, in which condition the main fat digestive and absorptive portions of the intestine are short-circuited. Extremely large quantities of fat may be present in the feces in tropical and nontropical sprue and in celiac disease, conditions commonly referred to as idiopathic steatorrhea. In these conditions the fat may constitute over 70 per cent of the dry weight of the feces, being present as both neutral fat and fatty acids, the latter usually predominating. Fatty diarrhea is usually the most constant manifestation and is accompanied by a variety of apparently secondary deficiency symptoms, including macrocytic anemia and low serum calcium and phosphorus concentrations, with evidence of rickets, osteomalacia or tetany, dependent apparently upon impaired absorption of calcium, phosphorus and vitamin D and, at times, evidence of vitamin A deficiency. There is frequently also an associated disturbance of carbohydrate metabolism, characterized by increased glucose tolerance, as evidenced by an abnormally flat blood sugar curve following the ingestion of glucose. This feature may be of value in differentiating these conditions from pancreatogenous steatorrhea, as may also be the fact that the latter condition is usually characterized by the fecal excretion of excessive quantities of nitrogen (more than 3 gm. daily) on a fat-free diet. Similar findings with regard to fecal fat may be obtained in chronic pancreatitis or in the presence of obstruction of the pancreatic duct with the exception that in such cases the proportion of neutral fat is relatively high and that of fatty acids low. This disturbance of fat partition has been generally attributed to impaired fat digestion incident to diminution in the amount of pancreatic lipase in the intestine in disease of the pancreas (p. 487).

Fecal fat is increased in intestinal lipodystrophy (Whipple's disease), in which fatty diarrhea is accompanied by accumulation of extracellular fat in the small intestine and regional lymph nodes, dilatation of lacteals and mesenteric lymphatics, and, occasionally, chylous ascites. This condition simulates idiopathic steatorrhea.

Absorption of fat may be interfered with also in other conditions in which there is obstruction of intestinal lymphatic drainage, e.g., tuberculosis, lymphosarcoma, Hodgkins' disease, and leukemia involving the mesenteric lymph nodes.

The feces may also contain large quantities of fat (to over 70 per cent of the dry weight) in patients with obstruction of the common bile duct or with bile fistula. In the absence of associated marked pancreatic disturbance the relative proportion of neutral fat and fatty acids is usually essentially normal, the latter predominating. This phenomenon has been generally attributed to a disturbance of fat absorption due to the absence of bile salts from the intestine (p. 86). Recent studies indicate, however, that the absence of bile from the intestine may, in itself, cause comparatively little impairment of fat absorption, 65 to 70 per cent of the fatty acids of the diet being absorbed in patients with bile fistula.

It would appear, therefore, that, as under normal conditions, fecal fat in the absence of bile has, to a large extent, an endogenous origin.

In the light of these facts, abnormal variations in the amount and partition of fecal fat cannot be interpreted for purposes of differential diagnosis as readily as was formerly believed.

FAT IN URINE

Fat may appear in the urine of normal individuals following ingestion of a high fat diet (alimentary lipuria) or following administration of such substances as cod liver oil. Lipuria may also occur in association with the lipemia of diabetes mellitus and nephrosis, following fracture of long bones with injury to the bone marrow and also following extensive superficial injuries with crushing of the subcutaneous fat. It has been reported in subjects suffering from poisoning with such agents as phosphorus and alcohol. Fat in the urine may also be derived from fatty degeneration of leukocytes and epithelial cells, particularly in such conditions as pyelonephritis and nephrosis.

The terms ''chyluria'' and ''lymphuria'' are applied to conditions in which there is some obstruction to the flow of lymph in the thoracic duct, with consequent distention and rupture of the lymph vessels of the kidney or bladder, the fat content of the urine in such cases varying with the quantity of fat ingested. The urine in chyluria is definitely milky in appearance, whereas in lipuria it is rather opalescent. It must be remembered that fat appearing in the urine after catheterization may be due to contamination with the lubricant used in passing the catheter.

METABOLISM OF PHOSPHOLIPIDS

It seems probable that all tissues synthesize phospholipids, but at widely varying rates. In all tissues but one, these compounds are apparently synthesized, used, and degraded *in situ*. Liver is exceptional, in that a large proportion of the phospholipids synthesized in this organ is liberated into the plasma. As a matter of fact, liver is practically the sole source of plasma phospholipids, and is also the chief site of their further metabolism or degradation. Although there is every indication that the phospholipids play important roles in intermediary metabolism, the nature of these roles can scarcely be conjectured at this time.

Turnover. By ''turnover'' is meant the rate at which a given type of molecule is renewed in the organism. Turnover may be a measure of the rate of total synthesis or degradation of the molecule in question, or, as is frequently the case in isotopic-labeling experiments, it may merely indicate the rate at which one segment of the molecule ''exchanges'' with its free brethren in the ''metabolic pool.''

Whether measured by labeling the phosphate, fatty acids, or bases, the turnover of phospholipids exhibits the same relative rates in the various tissues; liver, intestine and kidney comprise the most active group, such organs as pancreas, adrenals and lungs having intermediate activity, and muscle and brain being in the slowest category.

Data obtained in experimental animals using labeled acetate indicate that, in liver and plasma, neutral fat turns over more rapidly than phospholipid, whereas the converse holds true in most other organs (including mesenteric lipid). Turnover rates are equal for fat and phospholipid in the carcass. The half-life of liver and plasma phospholipid is a matter of hours rather than days.

As might be anticipated, the turnover of phospholipids is markedly affected by dietary or metabolic influences. Fat feeding increases the turnover in liver and intestine, decreases that of the kidney, and has no effect on other organs. Experimental diabetes results in markedly increased turnover in liver and plasma, whereas other tissues are affected slightly or not at all. Administration of choline increases the rate of turnover of the lecithin fraction of liver.

Catabolism. There is little information concerning the processes of breakdown of phospholipids in the tissues. The initial reactions are presumably hydrolytic, similar to those taking place in the digestive tract. Various "lecithinases" (p. 88), which are not specific for lecithin, are found in animal tissues. The liberated components are then free to pursue their individual metabolic pathways.

METABOLISM OF STEROLS AND BILE ACIDS

Of the many sterols in nature, cholesterol is the only one which is of any great significance in animal metabolism. (The products of ultraviolet irradiation of the plant sterol, ergosterol, are absorbed through the intestine and certain of them have vitamin D activity in animals.) It has been known for some time that the animal organism can synthesize cholesterol, although the mechanism was completely obscure until quite recently. With the aid of isotopic labeling, small fragments such as acetic acid and acetoacetic acid have been shown to be precursors of the sterol molecule.

In terms of total output, the liver is probably the major site of synthesis of cholesterol. Active synthesis occurs also in the adrenals, spleen, intestinal mucosa, red cell, bone, heart, omentum, muscle, skin, lungs, kidney, gonads, and perhaps in all tissues. It is of interest in connection with atherosclerosis that cholesterol can be synthesized by the arterial wall. Brain, which has a very high concentration of cholesterol, synthesizes the sterol at a very slow rate, if at all, in the adult. From isotopic labeling studies, it has been calculated that the "half-life" of a serum cholesterol molecule in man is eight days.

The rate of synthesis of cholesterol by the liver is influenced by a number of factors. It is, for example, inversely related to the supply of dietary cholesterol, which may be regarded as a homeostatic mechanism. The rate is depressed in the hypophysectomized animal, and frequently increased in the diabetic, matters which are discussed elsewhere (p. 75). The level of cholesterol in the plasma is influenced by the functional status of the thyroid (p. 121).

The relationships between the cholesterol contents of liver, plasma, and extrahepatic tissues resemble to a considerable degree the situation which obtains in the case of the phospholipids (p. 98). Liver is practically the sole source of plasma cholesterol, and it is also the main depository of cholesterol already present in the blood. Extrahepatic tissues synthesize their own supplies of cholesterol, liberate practically none into the blood, and draw upon the latter source to only a very slight extent.

Esterification of cholesterol occurs in the intestinal wall, liver, and in certain other tissues (e.g., the adrenal cortex). The liver is the chief source of esterified as well as of free cholesterol of the blood plasma, a fact of importance in connection with changes in plasma cholesterol in hepatic disease.

Catabolism and Excretion of Cholesterol; Conversion to Bile Acids. The general pathways of catabolism of cholesterol and the routes of excretion of the waste products are indicated in Figure 15. For convenience, the catabolism of cholesterol may be divided into two compartments, the excretory route via neutral sterols, and that involving the bile acids.

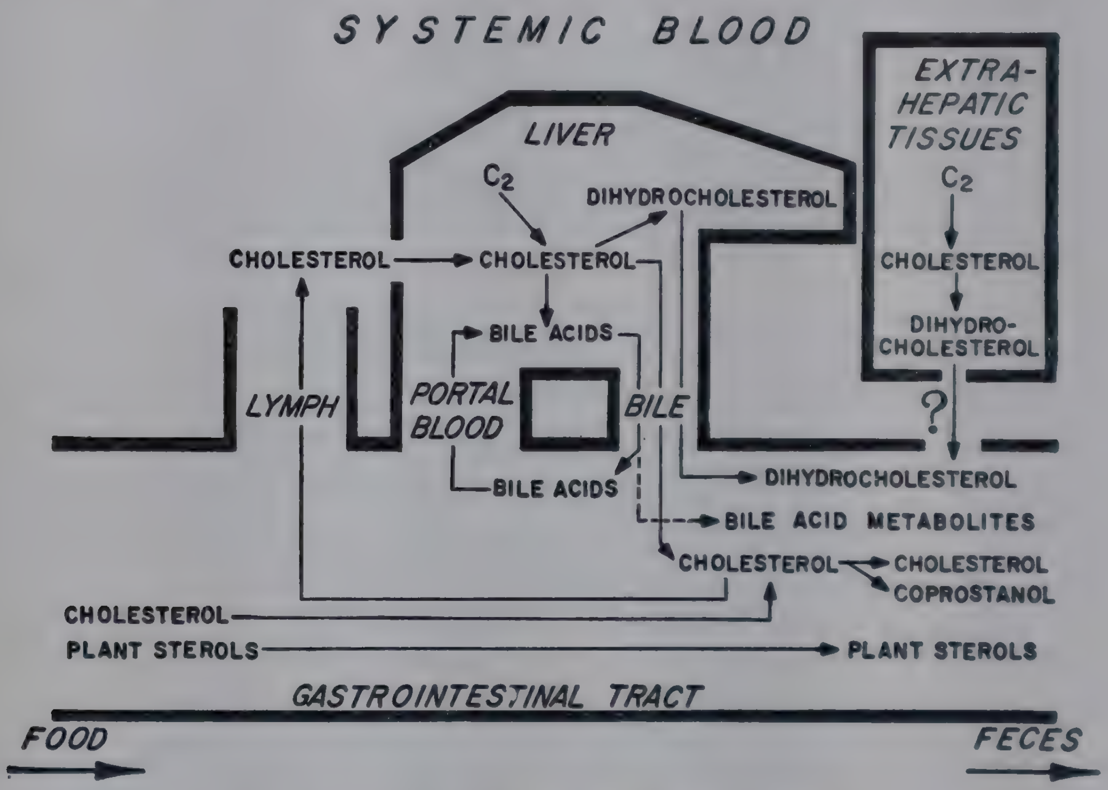

SYSTEMIC BLOOD
LIVER
EXTRA-HEPATIC TISSUES
C_2
DIHYDROCHOLESTEROL
C_2
CHOLESTEROL
CHOLESTEROL
CHOLESTEROL
BILE ACIDS
DIHYDRO-CHOLESTEROL
LYMPH
PORTAL BLOOD
BILE
?
BILE ACIDS
DIHYDROCHOLESTEROL
BILE ACID METABOLITES
CHOLESTEROL
CHOLESTEROL
COPROSTANOL
CHOLESTEROL
PLANT STEROLS
PLANT STEROLS
GASTROINTESTINAL TRACT
FOOD
FECES

Fig. 15. Over-all metabolism of cholesterol.

(a) Neutral Sterols. In the liver and other tissues, cholesterol is reduced to cholestanol (dihydrocholesterol). A certain amount of cholesterol and cholestanol is excreted through the intestinal wall. The major site of this excretion is said to be the large intestine. Cholesterol and cholestanol are excreted also by the liver, via the bile, into the small intestine. Biliary cholesterol is largely formed in the liver. The cholesterol thus excreted mixes with dietary cholesterol and may be partially reabsorbed by way of the lymphatics, mainly after preliminary esterification in the cells of the intestinal mucosa. Unabsorbed cholesterol and cholestanol (which cannot be absorbed) are found in the feces. A third neutral sterol excreted in the feces is coprostanol (coprosterol). Since neither this compound nor its possible immediate precursors have been found in the tissues, it is apparently formed by intestinal bacteria.

(b) Bile Acids. As determined by isotopic tracers, 10 to 20 per cent of exogenous cholesterol is accounted for in the neutral sterol fraction of bile and feces. The remaining 80 to 90 per cent is converted to bile acids (cholic acid and derivatives) in the liver and excreted via the bile into the intestine, from which the major fraction is reabsorbed. Unabsorbed bile acids are converted by intestinal bacteria into unidentified metabolites, which are excreted in the feces.

TRANSPORT OF LIPIDS

Phospholipids (chiefly lecithin), cholesterol, and neutral fat are the most abundant lipids in the plasma. There are small amounts of glycolipids and minute quantities of certain important hormones and vitamins of lipid nature. Normal values for the major plasma lipids are indicated in Table 1. Because of the heterogeneity of this group of substances and because different components have different metabolic origins, fates, and significance, it is necessary to consider them individually from these standpoints. Before doing so, however, certain general features of their state in the plasma should be reviewed. These have to do largely with recent observations concerning the intimate relationship between the plasma lipids and certain fractions of the plasma proteins.

State of Lipids in Blood: Lipoproteins. Practically all of the lipids of plasma are present as lipoprotein complexes. Electrophoretic studies of plasma protein fractions have disclosed the presence of two groups of lipoproteins, one migrating with the α_1-globulins, the other with the β_1-globulins. Ultracentrifugal analysis by the flotation technic has led to the detection of a hierarchy of lipoproteins of varying particle weight (p. 85).

The relatively low density groups (S_f 2-S_f 17) consist of components that are sufficiently discrete to be separable, whereas those above S_f17 are increasingly less capable of resolution. The major cholesterol- and phospholipid-bearing molecules are in the groups S_f17 and lower, whereas the major neutral fat-bearing molecules are in the higher categories. The upper limit of S_f 40,000 represents lipid particles (largely glycerides) known as chylomicrons. Under normal conditions (postabsorptive), the lipoproteins in the plasma of children (up to 15 years of age) fall almost exclusively into classes below S_f10. With increasing age, the concentration of the S_f 12–20 lipoproteins increases, reaching a maximum in men earlier (30 years of age) than in women (60 years). It is this class (S_f 12–20) to which attention has been directed by some in relation to the pathogenesis of atherosclerosis (p. 111). The β-lipoproteins, too, have been incriminated by some in this connection (p. 112).

The plasma lipoproteins contain cholesterol (free and esterified), phospholipids, neutral fat, and traces of the lipid-soluble vitamins and the steroid hormones. Transport of the latter substances in a water-soluble form may be an important function of the lipoproteins. The α_1-lipoprotein referred to above contains 12 per cent cholesterol, 21 per cent phospholipid, and 57 per cent protein, whereas the β_1-lipoprotein in flotation group S_f4 contains about 30 per cent cholesterol, 25 to 29 per cent phospholipid, and 23 to 25 per cent protein, in addition to traces of vitamins A, D, and E, and of estrogens. The chemical composition of the lipoproteins isolated by flotation appears to vary regularly with the density of the molecule; as one proceeds from S_f4 to S_f40,000, the content of cholesterol, phospholipid, and protein decreases gradually, as does the fraction of cholesterol present as ester (75% of the total cholesterol in class S_f4). The content of triglyceride, which is negligible up to class S_f17, increases progressively thereafter to a value of 75 to 85 per cent in the molecules of the highest S_f groups, which are therefore practically fat droplets (chylomicrons). The phospholipid : cholesterol ratio in the lipoproteins varies with the species of molecule in question. There are indications that the size of the lipoprotein aggregates is controlled to some extent by the over-all phospholipid : cholesterol ratio of the plasma, turbidity resulting from ratios below certain critical values.

During periods of fat absorption there is a transient elevation of molecular classes above S_f 60, often with the development of turbidity of the plasma. This turbidity is dispelled by administration of heparin ("clearing action"), which apparently reacts with some tissue factor (heart, lung) to produce a "clearing factor" which causes conversion of molecules above 30 S_f units to those below 30 S_f units. This is accomplished also by high molecular weight dextrans. The "clearing action" of heparin, like its anticoagulant action, is prevented by protamine (due presumably to salt formation between the acidic heparin and the basic protamine). However, there is evidence that the lipemia-clearing action of heparin is not related fundamentally to its anticoagulant effect, since the latter may be neutralized by agents (e.g., neutral red, toluidine blue) which have no influence upon the former.

Table 1. Lipids and Ketone Bodies in Blood Plasma (Fasting)

SUBSTANCE	CONCENTRATION (mg./100 ml.)
Total lipid	385–675 (530)
Neutral fat	0–260 (140)
Phospholipids	110–250 (165)
Lecithin	80–200 (110)
Cephalin	0–30 (10)
Sphingomyelin	10–50 (30)
Cholesterol	140–260 (200)
Ester	90–200 (145)
Free	40–70 (55)
Total fatty acids	110–485 (300)
Neutral fat × 0.95	
Phospholipid × 0.65	
Cholesterol ester × 0.43	
Ketone bodies (as acetone)	0.2–0.9

Postabsorptive Plasma Lipid Concentration. The range of normal variation in the concentration of plasma lipids is wide, even in the postabsorptive state (Table 1). Moreover, although the three major components, i.e., triglycerides (neutral fat), phosphatides (phospholipids), and cholesterol, frequently vary in the same direction, although not to the same degree, this is not always the case. Total fatty acids and neutral fat are particularly variable, even in the same individual. The cholesterol : phospholipid ratio is more stable and uniform, although its significance is not apparent. Little is known concerning the intrinsic mechanisms which regulate the equilibrium levels of the blood lipids. It is conceivable that the cholesterol : phospholipid ratio may be influenced, if not fixed, by the hepatic mechanisms for synthesis of the lipoproteins in which these molecules are found. There is no significant sex or racial difference in the concentrations of plasma lipids. Although there is no complete agreement on this point, there is evidence that "adult" patterns of blood lipid concentrations are established at an early age (first few days of life), and change but little, if at all, in later years in normal subjects. Low values for cholesterol and phospholipids have been reported in the newborn.

CHOLESTEROL. Cholesterol exists in the plasma in two forms, (1) free and (2) esterified (combined with fatty acids), both of which are incorporated in lipoprotein molecules (p. 101). Free cholesterol comprises about 20 to 40 per cent, and ester cholesterol about 60 to 80 per cent of the total of 140 to 260 mg./100 cc. This ratio is usually preserved with remarkable constancy even in the presence of wide variations in the total due to disease states that are not accompanied by disturbance of liver cell function. Although cholesterol is undoubtedly synthesized by a number of tissues, the liver is the main if not the only source of plasma cholesterol as well as the main site of its esterification (also intestinal mucosa, p. 88). This organ is therefore fundamentally concerned in the maintenance, not only of the level of cholesterol in the plasma, but also of the ester : free cholesterol ratio. It plays an important role, too, in the removal of this substance from the blood, and in its subsequent metabolism, as indicated by the fact that the bulk of exogenous cholesterol is recoverable as bile acids in the bile (p. 100).

PHOSPHOLIPIDS. The range of normal variation in plasma phospholipids is indicated in Table 1. Lecithin is the main component of this fraction, which, like other lipids, occurs in the blood plasma in association with certain globulins, as lipoprotein complexes (p. 101). It has been shown, by the use of isotope-labeling technics, that the plasma phospholipids, similarly to cholesterol, not only originate in the liver but are also removed from the blood and metabolized probably exclusively by that organ. It appears, therefore, that plasma phospholipids undergo their complete metabolic cycle within the liver, with a temporary sojourn in the blood stream, the purpose of which is not readily apparent. The constancy of the cholesterol : phospholipid ratio suggests that this (ratio) is of physiological significance. Present evidence renders untenable the formerly prevailing view that the plasma phospholipids serve as an important medium of transport of fatty acids between the liver and extrahepatic tissues.

TRIGLYCERIDES (NEUTRAL FATS). The variable concentration of neutral fat in the plasma in the postabsorptive state is indicated in Table 1. The components of this fraction, too, exist as lipoprotein complexes (p. 101), but, during the period of absorption of fats from the intestine, they occur temporarily also in free form in the plasma (chylomicrons, p. 85). In the postabsorptive state they represent mainly triglycerides mobilized from the fat depots, en route to the liver, where they undergo their primary metabolic changes. They may also include triglycerides from the liver, en route to the fat depots.

The role of growth hormone (p. 92), adrenocortical hormones (p. 91), and carbohydrate unavailability (p. 91) in stimulating mobilization of fat deposits is discussed elsewhere. It is apparent that fat enters the blood from the fat depots at an accelerated rate under a variety of conditions in which carbohydrate is either not provided in adequate amounts in the diet or cannot be utilized adequately because of hormonal or other abnormalities.

Influence of Food and Nutrition. After a mixed meal containing an abundance of fat, the plasma lipids begin to rise within two hours, reaching a maximum concentration in six to eight hours, subsequently returning to the basal level. The latter may not be reached within ten hours if a large amount of fat has been ingested. This postprandial hyperlipemia is due to the fact that the major portion of the dietary lipid is absorbed by the intestinal lymphatics and enters the systemic blood stream directly, via the thoracic duct (p. 87). The bulk of the increment is in neutral fat (30–150% increase), cholesterol and phospholipids making relatively minor contributions, increasing up to about 10 and 20 per cent, respectively, even in their absence from the diet. There is considerable individual variation in the lipemic response to meals. In certain normal subjects, the continual ingestion of a high-fat diet results in a cumulative effect, i.e., superimposition of the individual postprandial curves, with consequent elevation of the concentrations of total lipids and cholesterol in the plasma.

Under the conditions mentioned above, the plasma frequently exhibits turbidity, due mainly to the increase in neutral fat (chylomicrons). In abnormal states, e.g., in biliary cirrhosis, large independent increases in phospholipid (over 2000 mg./100 cc.) and cholesterol (over 1000 mg./100 cc.) may occur without turbidity of the plasma.

Because of the suggested relationship between hypercholesterolemia and atherosclerosis (p. 111), the influence of diet on the plasma cholesterol level has received considerable attention. It seems to have been established that, at least in normal subjects, the plasma cholesterol concentration is not influenced significantly by variations in the dietary intake of this substance over a rather wide range (0 to 700 mg.). However, a significant decrease may be produced in subjects with certain types of hypercholesterolemia by drastically reducing the total lipid intake, i.e., a diet of extremely low fat content, which is scarcely practicable and is probably undesirable. These facts are to be anticipated because of the demonstrated large capacity (1.5–2 gm. daily) of normal subjects for synthesizing cholesterol from acetate, that common denominator of organic metabolism. Moreover, it has been shown that cholesterol administration causes a decrease in the amount synthesized, so that the total provision of this substance varies but little in the presence of wide variations in exogenous supply. It should be pointed out that dietary deficiencies or imbalances that result in certain types of fatty liver are associated with hyperlipemia, including, at times, hypercholesterolemia, e.g., carbohydrate, pantothenic acid deficiencies (p. 106ff.).

There is no general correlation between the blood lipid levels and the amount of body fat, but chronic malnutrition and wasting diseases are often accompanied by subnormal levels of phospholipids and cholesterol. In the initial stages of starvation, or of carbohydrate deprivation, after depletion of the preformed glycogen stores (about 36 hours), hyperlipemia occurs as a result of increased mobilization of depot fat (p. 91). This may be accompanied by fatty liver (p. 107). If the fasting period is prolonged to the point of depletion of available fat stores, the concentration of plasma lipids may fall.

Influence of Age and Sex. Age and sex differences in lipoprotein fractions are considered elsewhere (p. 101). The plasma phospholipids are relatively low in pre-adolescence, rising to levels of 110 to 250 mg./100 cc. (lipid P = 4–10 mg.) in early adult life. The plasma cholesterol appears to be quite low at birth, about 50 mg./100 cc., increasing rapidly within the first few days to about 20 to 25 mg. below the average normal adult level, where it remains until adolescence.

The significance of much of the reported data on sex and age differences in plasma cholesterol is difficult to evaluate because of differences in analytical technics and the wide range of normal variation. The following conclusions seem to be warranted on the basis of recent critical statistical analyses: (1) Between 17 and 30 years of age, the plasma cholesterol concentration shows an average increase of about 2 mg./100 cc. per year, with no significant sex difference. (2) It continues to increase in men, reaching a maximum in the sixth decade. No satisfactory data are available on women in the older age groups. (3) Values above 300 mg./100 cc. occur in 1 per cent of normal men over 35 years of age, above 300 mg. in 5 per cent of those 45 to 60 years old, and above 320 mg. in 1 per cent of those 45 to 70 years old.

Pregnancy. The concentrations of cholesterol, phospholipids, and neutral fat increase during pregnancy. The maximum increase in cholesterol is reached at about the thirtieth week, the average increment in the free fraction being about 25 per cent and in the ester fraction about 5 per cent. The normal level is restored about eight weeks postpartum. The phospholipids increase to about the same extent as cholesterol, with an occasional additional rise during lactation.

The cause of the increased lipidemia in pregnancy is not known. Endocrine factors may be involved. The blood lipids, including cholesterol, increase in female birds during puberty and the period of egg production. This phenomenon can be produced by administration of estrogen to male and nonlaying female birds. However, attention should be directed to the observation that estrogen caused a decrease in the plasma cholesterol in atherosclerotic subjects, due mainly to decrease in the β-lipoprotein fraction (p. 113).

DEPOSITION AND STORAGE OF LIPIDS

Role of Liver in Lipid Metabolism. Although incidental mention has been made of the role of the liver in various aspects of lipid metabolism, it seems desirable to summarize here the pertinent information on this point, since the phenomena to be discussed in this section involve the liver primarily.

(*a*) The liver is the chief site of synthesis of fatty acids from carbohydrate, and of cholesterol from acetate fragments.

(*b*) It is probably the sole source of bile acids.

(*c*) The phospholipids and cholesterol (free and esterified) of the plasma, and the lipoproteins in which they are incorporated, are synthesized by the liver.

(*d*) In the other direction, the liver is the organ chiefly concerned in removal of phospholipids, cholesterol, and probably also the lipoproteins from the plasma.

(*e*) It is the major site of degradation of fatty acids of dietary or depot origin, when the physiological state of the body calls on fat for the major provision of energy.

(*f*) It is the only physiologically significant site of formation of ketone bodies.

(*g*) The liver is one of the poles of the "liver-depot axis," along which fat is transported in one direction or the other in response to physiological needs. In this connection, the level of fat in the liver at a given time reflects the current status and net direction of the mobilization mechanisms.

Fatty Liver and Lipotropism. The amount of lipid in the liver at any given time is the resultant of several influences, some acting in conjunction with, some in opposition to others. (Normal total lipid about 4 per cent, three-fourths of which is phospholipid, one-fourth neutral fat.) Factors that tend to increase the fatty acid content of the liver are: (1) the synthesis of fatty acids in that organ from carbohydrate and protein, (2) influx of dietary lipid, and (3) mobilization of fat to the liver. Decrease in liver fatty acids results from: (1) mobilization of fat to the depots from the liver, (2) passage of cholesterol esters and phospholipids into the blood, (3) degradation of the fatty acids within the liver itself. Normal levels of lipid in the liver are the result of maintenance of a proper balance between these factors. A relative increase or decrease in the rate of one or other of these processes can result in the accumulation of abnormal quantities of lipid in the liver, so-called "fatty-liver." On this basis, five types of fatty liver can be distinguished, in theory at least, due to the following causes: (1) overfeeding of fat; (2) oversynthesis of fat from carbohydrate; (3) overmobilization from depots to liver; (4) undermobilization from liver to depots; (5) underutilization in the liver. Although it is doubtful whether all of these types have been observed in uncomplicated form, a summary of the probable general features characteristic of each is presented in Table 2.

TYPE 1. Although fatty livers of pure Type 1 can be produced in experimental animals, they are frequently deliberately aggravated by the imposition of conditions conducive to the development of Types 2 or 4. The lipids deposited in Type 1 reflect the composition of the dietary lipid, as would be expected. The "cholesterol fatty liver" may belong to this category, although it is usually considered in another context (v. infra, Type 4).

TYPE 2. Oversynthesis from carbohydrate can result from forced feeding; it is more generally produced experimentally by the administration of excessive amounts of certain B vitamins (thiamine, biotin, riboflavin) or cystine, which seem to stimulate the appetite. Increased consumption of fat as well as carbohydrate lends to this type of fatty liver certain of the characteristics of Type 1. The situation is even more complex, however. The increase in general metabolic activity occasioned by the inclusion of large quantities of B vitamins or cystine in the diet results in a greater demand for certain factors, such as inositol and choline (v. infra, Type 4). The consequent relative deficit in these factors actually produces fatty livers of Type 4.

TYPE 3. Fatty liver of this type is referred to by some as "physiological" fatty liver, because it represents merely an exaggeration of a normal process, the mobilization of fat from the depots to the liver. The normal proportions among the various types of lipids in blood and liver are maintained: all are equally elevated in concentration. Fatty livers of this type develop in conditions involving greatly increased utilization of fat as fuel, which is the physiological equivalent of saying all conditions in which there is interference with the oxidation of carbohydrate. Such conditions exist in: (1) diabetes, human or experimental, of the hypoinsulin, hyperpituitary, or hyperadrenocortical types; (2) "pseudodiabetes" induced by phlorizin; (3) starvation; (4) carbohydrate deprivation. The fat which accumulates in the liver is derived from the depots, the fat content of which de-

Table 2. Classification of Fatty Liver

TYPE	EFFECT ON BLOOD LIPIDS	EFFECT ON LIVER LIPIDS	EFFECT ON DEPOT LIPIDS	IMMEDIATE CAUSE	CURATIVE AGENTS* (LIPOTROPIC FACTORS)
1. Overfeeding	None	Increase	Increase	Excessive fat in diet	Choline and precursors or substitutes
2. Over-synthesis	None	Increase	Increase	Excessive carbohydrate, cystine, B vitamins in diet	Choline and precursors or substitutes
3. Overmobilization to liver	Increase, normal pattern	Increase, normal pattern	Decrease	Carbohydrate deprivation (dietary, hormonal)	
4. Undermobilization from liver	Decrease, especially in phospholipid and cholesterol	Increase in fat and cholesterol; decrease in lecithin	Decrease	Deficiency of: Essential fatty acids	
				Pyridoxine	Inositol
				Pantothenic acid	Choline and precursors or substitutes
				Choline (direct or indirect)	Choline and precursors or substitutes, lipocaic
				Excess of: Diet cholesterol	Choline and precursors or substitutes, plus inositol or lipocaic
				Biotin	Inositol; lipocaic
5. Underutilization	Increase in phospholipid and cholesterol Decrease later, if severe	Increase	Increase; decrease ?	Deficiency of pantothenic acid; hepatotoxic agents	Choline and precursors or substitutes

* Other than direct removal of cause.

creases accordingly. In addition to fatty liver, this condition is characterized also by ketosis and, in advanced cases, acidosis.

TYPE 4. Fatty liver of this type has been differentiated from the preceding type by being designated "pathological." It is accompanied by a decrease in plasma lipids (hypolipemia) which affects mainly the phospholipids and cholesterol esters. The pattern of liver lipids is also abnormal; fat and cholesterol esters are especially increased, whereas lecithin is present in less than the usual proportion. Fatty livers of this type may eventuate in cirrhosis of the liver and there may be associated hemorrhagic lesions in the kidneys. They appear to be caused by agents or conditions which produce either an absolute or a relative deficiency in certain of the ingredients used by the liver for synthesis of phospholipids, viz., choline, inositol, and the poly-unsaturated fatty acids. The resulting condition appears to reflect some sort of blockage of metabolism of fat in, or its movement through the liver. According to certain investigators, the degradation of fatty acids is impaired; the majority opinion holds that the integrity of phospholipid metabolism in the liver is necessary for the mobilization of fat from that organ.

Agents (e.g., choline, methionine, betaine, and inositol) which have the apparent effect of facilitating the removal of fat from the liver are said to be "lipotropic," the phenomenon itself being called "lipotropism." Antagonistic agents and the converse condition are "antilipotropic" and "antilipotropism," respectively.

Fatty livers of Type 4 may be roughly classified according to the causative agent or phenomenon:

(*a*) *Deficiency of Essential Fatty Acids.* The fatty livers due to deficiency of the essential fatty acids are cured only by the reintroduction of these substances into the diet. Since the phospholipids of the liver are characterized by a relatively high content of poly-unsaturated fatty acids, it is surmised that a shortage of the latter substances results in impairment of synthesis or turnover of the former.

(*b*) *Imbalance of B Vitamins.* Deficiencies of pyridoxine and pantothenic acid, and excessive amounts of biotin in the diet, give rise to fatty livers, the etiologic factor of which is in dispute. It is possible that the fatty liver of pantothenic acid deficiency properly belongs to Type 5 rather than 4, since a shortage of CoA (of which pantothenic acid is a part) might be expected to result in impairment of the degradative mechanisms for fatty acids. Lack of pyridoxine and a surplus of biotin are said to elicit a greater demand upon the supply of inositol, thus supposedly interfering with the synthesis of inositol-containing phosphatides. Although the fatty livers in these conditions respond well to inositol, this explanation leaves much to be desired.

(*c*) *"Cholesterol" Fatty Livers.* Fatty livers resulting from administration of excessive amounts of cholesterol are sometimes regarded as belonging to Type 4, although they may perhaps as readily fit the requirements of Type 1. One explanation advanced for the former classification is that cholesterol may compete with phospholipids for the polyunsaturated fatty acids, for the acids found in cholesterol esters are, on the average, even more highly unsaturated than those of phospholipids.

(*d*) *Choline Deficiency.* The remaining fatty livers of Type 4 are due, more or less directly, to a deficiency of choline. Since choline is synthesized by the successive methylation of ethanolamine, it is true that induced deficiencies of the methyl group produce fatty livers, solely, however, because of the resulting shortage of choline.

Animals maintained on diets deficient in the usual sources of preformed methyl groups (choline, methionine, betaine), and also in the vitamins necessary for synthesis of methyl groups from appropriate precursors (folic-folinic acid group, B_{12}), develop fatty livers of Type 4. These fatty livers are cured by the methyl donors mentioned above, by choline itself or its proper analogs, and, as shown recently, by folic acid.

A deficiency of choline can be induced also by inclusion in the diet of compounds which will compete with ethanolamine for available methyl groups. Nicotinic acid or amide and guanidoacetic acid, for example, are methylated in the body to N^1-methylnicotinamide and creatine, respectively. Administration of greatly excessive quantities of these compounds depletes the supply of methyl groups available for the synthesis of choline, and fatty liver results.

Pancreatectomized animals maintained adequately with insulin develop fatty livers, not of the overmobilization type characteristic of diabetes, but of Type 4. The condition is alleviated by the administration of raw pancreas. According to one school of thought, "lipocaic," a purported internal secretion of the pancreas, is the lipotropic agent responsible. The majority believe that an external secretion of the pancreas is involved, probably a proteolytic enzyme which specifically facilitates the liberation of methionine from the proteins of the food.

Other substances exert a lipotropic action, the mechanism of which has not been explained, e.g., estrogens and threonine.

TYPE 5. As suggested previously, it is possible that the fatty livers of pantothenic acid deficiency are of this type, i.e., underutilization. Those due to hepatotoxic agents (chloroform, carbon tetrachloride, phosphorus) may also belong to this group, although they present in addition certain of the features of Type 4. The initial elevation of the serum lipids (during which time the depots may have an increased content of fat) may be due to impairment of the mechanisms for the degradation of fatty acids, but not of the mobilization of fat from the liver to the depots. The second, or hypolipemic phase (during which time the depots are probably somewhat depleted), may reflect interference with the mobilizing mechanisms of the liver. In support of the probably composite nature of the pathogenesis of this type of fatty liver, it may be mentioned that it is possible to counteract or mitigate with choline or methionine the fatty infiltration due to the hepatotoxic agents, if the therapeutic agent is given promptly after administration of the toxic agent, or better, prophylactically.

LIPOIDOSIS, XANTHOMATOSIS

These designations are applied to a group of conditions characterized by infiltration of various tissues by reticuloendothelial cells containing large amounts of lipid material ("foam" cells). The nature of the lipid in these so-called xanthomatous lesions differs in different conditions within this category: cholesterol in the Schüller-Christian syndrome; a galactose-containing cerebroside, resembling kerasin, in Gaucher's disease; sphingomyelin in Niemann-Pick's disease, and a special galactoside (Substance X) in the Tay-Sachs syndrome. In addition to these primary (essential) lipoidoses, implying some inherent metabolic abnormality in the affected reticuloendothelial cells, there is a group of secondary xanthomatoses, in which the xanthomatous lesions are believed to develop as a result of pre-existing protracted hypercholesterolemia. Whether or not this distinction is valid may be open to question, and no conclusion as to the pathogenesis of these abnormalities of lipid storage is justified at the present time.

In those varieties characterized by lipids other than cholesterol, no specific significant abnormality has been observed in the plasma lipids. In those in which cholesterol is the characteristic lipid, to which the designation of "xanthomatosis" is restricted by Thannhauser, the plasma cholesterol may or may not be increased. The following classification has been proposed:

I. Primary (Essential) Xanthomatosis

A. Normocholesterolemic Type. This includes: the majority of cases of the Schüller-Christian syndrome; xanthomata of the lungs and pleura, brain and dura; xanthomata disseminata of the skin; scattered osseous and visceral (liver, spleen) xanthomata (also present in B).

B. Hypercholesterolemic Type. This includes: occasional cases of the Schüller-Christian syndrome; scattered visceral (liver, spleen) and osseous xanthomata (also present in A); xanthomatous lesions of bile ducts, with biliary cirrhosis; xanthomata of tendons, endocardium, and vascular endothelium; xanthomata tuberosa and plana (cutaneous).

C. Combined Types A and B.

II. Xanthomatosis Secondary to Hyperlipemia

1. Idiopathic hyperlipemia.
2. Hyperlipemia occurring in diabetes mellitus, biliary obstruction, nephrosis, hypothyroidism, glycogen storage disease, etc.

As indicated above, although such a classification may be useful for descriptive purposes, it may not necessarily reflect accurately differences in pathogenesis of the xanthomatous lesions. The occurrence of hypercholesterolemia in these conditions is discussed elsewhere (p. 113ff.).

ABNORMALITIES OF PLASMA LIPIDS

Although variations occur in the plasma fat and phospholipid concentrations in certain abnormal states, determinations of these factors have not been made commonly, clinically, due mainly to technical difficulties and to the fact that alterations in cholesterol, which usually occur simultaneously, can be demonstrated much more readily. In recent years, demonstration of the fact that lipids are transported in the plasma in combination with proteins, i.e., as lipoproteins, different fractions of which contain different proportions of cholesterol and phospholipids (p. 101), has stimulated investigation of such factors as (*a*) the concentration of S_f 12–20 lipoprotein molecules (p. 101), (*b*) the ratio of alpha- to beta-lipoproteins, (*c*) the cholesterol: phospholipid ratio, and (*d*) the chylomicron count (turbidity) in clinical disorders. Most of these studies are as yet inconclusive, insofar as their specific diagnostic, prognostic, or fundamental significance is concerned.

Attention will be directed here mainly to observations on the plasma lipoproteins and cholesterol. Clinically significant abnormalities in fat and phospholipids will be mentioned incidentally. In general, with few exceptions, changes in plasma fat, phospholipids, and cholesterol concentrations in disease occur in the same direction, although not necessarily to the same degree.

PLASMA LIPOPROTEIN ABNORMALITIES AND ATHEROSCLEROSIS

Reference is made elsewhere (p. 101) to the fact that the plasma lipids, including cholesterol and phospholipids, are combined with plasma proteins, mainly α- and β-globulins, to form so-called "giant molecules," i.e., lipoprotein complexes of high molecular weight (e.g., 1,000,000 or higher). Studies in this field are being conducted intensively, with particular reference to the possible role of plasma lipoprotein abnormalities in the pathogenesis of atherosclerosis. Although no definite conclusion can be reached as yet on this point, clinical interest in this field necessitates a brief review of the current status of this important problem.

For many years there have been attempts to relate atherosclerosis to some abnormality in plasma lipids, particularly to hypercholesterolemia. Belief in the possibility of such a relationship arose in part from the observation that the lipid composition of the intima of arteries resembles that of the blood plasma, suggesting that the lipids may pass from the latter to the former. Moreover, it was shown that atherosclerosis develops in rabbits in which hypercholesterolemia had been induced by feeding cholesterol. The applicability of this observation to atherosclerosis in man was regarded as questionable, inasmuch as the rabbit is a herbivorous animal and normally ingests no cholesterol. However, recently, this disease has been observed in chicks (omnivorous) and dogs (carnivorous) with experimentally induced hypercholesterolemia. It is also true that the incidence of atherosclerosis is unusually high in certain conditions in which the plasma cholesterol concentration tends to be elevated for prolonged periods, e.g., diabetes mellitus, nephrosis, and familial hypercholesterolemia (lipemia). On the other hand, there have been many observations that suggest the lack of relationship of cause and effect between hypercholesterolemia and clinical atherosclerosis (p. 118).

Improvement in our understanding of the state in which lipids exist in the blood, i.e., as lipoproteins, has opened new approaches to the study of possible metabolic abnormalities that may consist in alterations in the plasma lipid distribution pattern rather than in the absolute concentration of individual lipid components. Two main types of abnormality in this direction have aroused considerable interest, viz., (*a*) increase in S_f 12–20 lipoproteins, and (*b*) increase in β-lipoproteins.

Increase in S_f 12–20 Lipoproteins. Employing the ultracentrifugal flotation technic (p. 85), Gofman and his associates have obtained data which suggest that the incidence of atherosclerosis, both experimental and clinical, is correlated with the concentration of the S_f 12–20 class of plasma lipoproteins. He has reported this to be increased in subjects with diabetes mellitus, hypertension, hypothyroidism, nephrosis, and coronary occlusion. Although the concentration of this class of molecules tends to rise with increasing cholesterol levels, this relationship was not found to be consistent. According to these authors, S_f 12–20 lipoproteins occur normally in only low concentration, if at all, in the plasma of young subjects, and more commonly in males than in females. The concentration was found to increase progressively with age, more rapidly in males, the quantitative difference between men and women tending to disappear after 40 years of age. Restriction of dietary fat and cholesterol was stated to result in significant lowering of the S_f 12–20 lipoprotein concentration and also the rate of recurrence of myocardial infarction.

Technical difficulties have limited the general applicability of studies of this type, but contradictory data are already emerging. Various authors have reported: (1) that the S_f 12–20 lipoproteins are normal in two-thirds of diabetic subjects with atherosclerosis but without renal complications (glomerulosclerosis), which increase the incidence of abnormality in this fraction; (2) that the mean values are essentially normal in males with hypertension or coronary artery disease, but may be elevated in women with these conditions; (3) that although the range of concentration of the S_f 12–20 class of lipoproteins may be wider than normal in subjects with atherosclerosis, there is broad overlapping with normal values; (4) that abnormalities in the S_f 12–20 molecules show no closer correlation with atherosclerosis than does hypercholesterolemia.

Beta-Lipoprotein Increase. By means of the Cohn fractionation procedure (p. 143), it has been shown that virtually all of the plasma cholesterol and phospholipid is present in two fractions, designated α- and β-lipoproteins. The S_f 12–20 molecules are probably included in the latter category (i.e., beta). One of the significant differences between these two fractions is that the phospholipid: cholesterol ratio is relatively high in the α-lipoproteins as compared with the β-lipoproteins. This difference may, according to some, form the basis for significant differences in molecular aggregation and biological actions of these molecules, the hydrophilic phospholipids presumably facilitating the solubilization of the hydrophobic cholesterol. It has been reported (Barr) that there is a relative and absolute increase in the concentration of β-lipoproteins, and, therefore, a decrease in the α:β ratio, in subjects with atherosclerosis and also those with predisposing conditions, e.g., nephrosis and diabetes mellitus. In contradistinction, hyperlipemia in biliary cirrhosis, in which the incidence of atherosclerosis is not unduly high, was found to be due usually mainly to an increase in the α-lipoprotein fraction.

The clinical significance of this biochemical abnormality was supported further by the observations: (1) that the $\alpha:\beta$ ratio is fairly constant in normal young subjects; (2) that the β-lipoproteins are relatively lower in young women (18–35 years) than in men; (3) that this sex difference disappears with advancing age (40–65 years). The possible importance of this sex difference, corresponding to the difference in incidence of atherosclerosis, was emphasized by preliminary studies indicating that the β-lipoproteins decrease after administration of estrogen and increase (in animals) after administration of androgen.

Effect of Heparin. Reference is made elsewhere to the "lipemia-clearing" action of heparin (p. 102). This effect in decreasing the turbidity of lipemic plasma is accompanied by a shift in the lipid distribution pattern toward molecules of the lower S_f classes. Moreover, the abnormal plasma lipoprotein pattern in atherosclerotic subjects is shifted toward normal. A similar shift from β-lipoproteins to α-lipoproteins, i.e., toward a more normal pattern, has been observed in subjects with idiopathic hyperlipemia and with primary hypercholesterolemic xanthomatosis after administration of heparin. In the latter disorder, the plasma is not abnormally turbid. It has been suggested that regulation of the plasma lipoprotein pattern may be an important function of heparin, and that deficiency in this factor, or in some agent with similar properties, may underlie the presumed lipid metabolic (or transport) defect in the conditions under consideration.

The possible importance of this action of heparin is indicated by reports that it protects against the development of atherosclerosis in cholesterol-fed rabbits and relieves anginal pain in patients with coronary artery disease. It is pertinent in this connection to mention the observation that hyperlipemia, i.e., turbidity, and increase in the higher S_f classes, are accompanied by significant slowing of the circulation in various capillary areas. This abnormality, too, is corrected by administration of heparin.

As indicated above, studies of plasma lipids in atherosclerosis have been directed toward determination of: *(a)* cholesterol concentration; *(b)* phospholipid : cholesterol ratio; *(c)* concentration of S_f 12–20 molecules; *(d)* ratio of alpha- to beta-lipoproteins; *(e)* chylomicron content (turbidity). The relation of cholesterol to this disease is discussed elsewhere (p. 118). Conflicting observations with regard to the lipoproteins in this connection make it hazardous to ascribe diagnostic, prognostic, or pathogenetic significance to abnormalities in these factors at the present time. Studies in experimental animals are suggestive of an important relationship. However, one of the fundamental difficulties in evaluating statistical data in man resides in the fact that atherosclerosis cannot always be detected readily and, therefore, absolutely normal values for these plasma components cannot be established unequivocally. This point is emphasized by the observation that over 75 per cent of young U. S. Army soldiers (average age 22 years) killed in Korea had coronary artery atherosclerosis.

HYPERCHOLESTEROLEMIA

Diabetes Mellitus. The well-recognized occurrence of hyperlipemia in diabetes mellitus has attracted considerable attention (p. 74). Despite its chemical individuality, it has been shown that the concentration of plasma cholesterol in this condition tends to parallel approximately that of the total fatty acids of the blood, changes in the latter being reflected more or less accurately in the former. Early investigators of this problem came to the conclusion that diabetes mellitus is accompanied by a marked increase in plasma lipids, including cholesterol, and that this increase is progressive with the seriousness of the condition. Many have emphasized the importance of the plasma cholesterol concentration as an index of lipid metabolism and of prognosis in patients with diabetes. Patients with normal blood sugar values following treatment, but with persistent hypercholesterolemia, have been found to be more susceptible to relapse following slight dietary indiscretion or intercurrent illness, to severe complications (neuritis and gangrene) and to insulin refractoriness in the presence of complicating infectious processes. Changes in plasma cholesterol appear to be less marked in diabetes in children. Hypercholesterolemia is observed rather consistently in the presence of diabetic coma, and cases of progressive diabetes present values that are somewhat higher than those of decreasing severity. However, normal cholesterol values are usually obtained in uncomplicated cases in children and appear to bear no relation to the incidence of complications.

Occasionally, subnormal values for plasma cholesterol may be obtained in patients with advanced grades of diabetes. This finding, which is considered elsewhere (p. 122), is of serious prognostic significance.

In the great majority of cases the hypercholesterolemia of diabetes mellitus is due to an approximately proportionate increase in both free and ester fractions. The normal ratio is usually maintained even though the total cholesterol concentration may be increased 400 to 500 per cent. There is some evidence, however, that in cases of long duration, with extensive arteriosclerosis, the proportion of cholesterol esters may be increased 10 to 15 per cent above normal. Some observers believe that this observation is of significance in connection with the purported relationship between hypercholesterolemia and arteriosclerosis.

Improved understanding of (1) the role of the liver in the synthesis of plasma cholesterol, and (2) the metabolic interrelations of fatty acids and carbohydrates, permits a more satisfactory explanation of the hypercholesterolemia of diabetes than was formerly possible. The underlying mechanism is the same as that operating to produce excessive ketogenesis (p. 94). "Acetate," arising from carbohydrate (via pyruvate) (p. 8), amino acids (p. 138), and fatty acids (p. 90), can take several metabolic paths, under normal conditions, one of which is cholesterol synthesis (p. 99). In diabetes mellitus, there is, in the liver, a diminished supply of oxalacetate, one molecule of which is necessary to effect the oxidation of a molecule of acetate to CO_2 and H_2O. Moreover, because of the existing insulin deficiency, acetate cannot be utilized adequately for synthesis of fatty acids; it is simultaneously being produced in increased amounts from the stimulated catabolism of fatty acids (p. 90). The situation, therefore, is one in which increased amounts of "acetate" are deprived of two major metabolic pathways, and, in the liver, are diverted in excess into ketogenesis and plasma cholesterol synthesis.

There is evidence that hemoconcentration may contribute to the increase in the concentration of cholesterol, phospholipids, and neutral fat in severe diabetic acidosis. Dehydration in this condition may be accompanied by an increase in the plasma protein concentration, which falls, rapidly, with the cholesterol, during recovery.

There is no definite parallelism between cholesteremia and glycemia, glycosuria, ketonuria or acidosis in diabetes. Extremely high figures have been reported, a few being above 1000 mg. per 100 cc. of plasma, several above 500 mg. and many above 300 mg. per 100 cc. The administration of insulin is usually followed by a diminution in plasma cholesterol in diabetes. The determination of plasma cholesterol may serve as a valuable measure of the efficacy of therapeutic procedures in diabetes and as an indication of the necessity for the continuation of insulin therapy after the restoration of the normal blood sugar level and the disappearance of glycosuria.

Anesthesia. An increase in plasma cholesterol occurs rather consistently during and following ether and, less constantly, chloroform narcosis. Ether anesthesia is accompanied by a state of hyperglycemia which frequently tends to be proportional to the degree of hypercholesterolemia. It has been found that both of these phenomena may be prevented by the administration of insulin, which suggests that the simultaneous disturbances in cholesterol and carbohydrate metabolism incident to ether anesthesia are fundamentally related to one another.

The Nephrotic Syndrome. Hypercholesterolemia is a rather constant manifestation of the nephrotic syndrome (amyloid nephrosis; chronic active glomerulonephritis, p. 625). This increase is associated with retention of other lipids in the blood, chiefly neutral fat, but also phospholipids. Values for plasma cholesterol as high as 2200 mg. per 100 cc. have been reported and figures of 500 to 700 mg. are not unusual. The total lipids often exceed 2 gm./100 cc. Although some investigators of this problem have reported an increase in the proportion of cholesterol esters (80–90 % of the total) in this condition, the majority have failed to find any alteration in the distribution of cholesterol in the plasma. This hypercholesterolemia is associated with increased elimination of cholesterol in the urine and with its deposition in the renal tubular epithelium. An increase in plasma cholesterol is also found occasionally in cases of acute glomerulonephritis. It has been reported that, in the nephrotic syndrome, the major lipid increment occurs in the β-lipoprotein fraction, with consequent increase in the cholesterol : phospholipid ratio, and the S_f 12–20 class of lipoproteins. According to some, this is of significance in relation to the high incidence of atherosclerosis in this condition.

The significance of this phenomenon and the factors responsible for its development are not clearly understood. Its association with a diminished concentration of serum protein and particularly serum albumin suggests the possibility that both phenomena may be concomitant if not related features of some underlying metabolic disorder. Although edema, hypoproteinemia and hypercholesterolemia are usually present concomitantly in this condition, there is no constant quantitative relationship between the degree of cholesterolemia and the other two phenomena. A lack of fundamental dependence of the hyperlipemia on the hypoalbuminemia is indicated by the fact that the former does not occur in the presence of low plasma albumin levels due to nutritional causes (e.g., starvation, hepatic disease, celiac disease), or induced in animals by plasmapheresis. In fact the plasma cholesterol may be decreased under such circumstances. It is of interest in this connection that the diuresis induced in nephrotic patients by administration of ACTH (adrenocorticotrophic hormone) or cortisone is often accompanied by a decrease in plasma cholesterol and lipemia (also increase in plasma albumin). This does not occur with diuresis induced by other means, e.g., administration of urea. The mechanism of production of this effect is not known.

Some attribute the hypercholesterolemia of the nephrotic syndrome to some defect in the mechanism which ordinarily removes cholesterol from the blood or to some disturbance which causes excessive mobilization of lipids from the fat depots. Although an increase in other plasma lipids is observed commonly in all types of chronic glomerulonephritis, hypercholesterolemia occurs usually only in the presence of the nephrotic syndrome complicating the nephritic process. In the absence of this syndrome, the plasma cholesterol concentration is usually within normal limits in patients with chronic glomerulonephritis. Subnormal values may be obtained in patients in the terminal states of this condition (p. 122).

Hepatic and Biliary Tract Disease. The plasma cholesterol concentration is usually increased in jaundice due to uncomplicated common duct obstruction, the degree of hypercholesterolemia increasing with that of hyperbilirubinemia and returning to normal with release of the obstruction. In about 50 per cent of such cases the cholesterol esters increase concomitantly with the total cholesterol, the normal ratio between the free and the ester fractions being maintained; in the remainder, the increase occurs largely or entirely in the free fraction. The basis for this difference is not clear. In certain instances it is probably the presence of superimposed hepatocellular damage (see below). In others, however, this is apparently not the case. Changes in phospholipid concentration roughly parallel those in free cholesterol.

Hypercholesterolemia may occur also in cases of biliary cirrhosis, often with an increase in phospholipids. In some instances, apparently, the increase occurs mainly in the beta-lipoprotein fraction, in others in the alpha.

The mechanism of production of the increase in plasma cholesterol in obstructive jaundice is not known. The former view that it is a retention phenomenon is generally regarded as untenable, since the intestine, and not the bile, is the chief avenue of excretion of cholesterol. However, this opinion may require modification in the light of present belief that the cholesterol of both the bile and the blood plasma originates in the liver cells, in contrast to the former view that the hepatic cells remove cholesterol from the plasma and excrete it in the bile. As indicated elsewhere (p. 100), although cholesterol is removed from the plasma by the liver, almost exclusively, it apparently is excreted in the bile largely as bile acids, not as cholesterol. This conversion to bile acids (cholic acid) occurs in the hepatic cells. Evidence has been presented which suggests that the hypercholesterolemia of biliary obstruction is dependent, in some manner, on the associated increase in bile acids in the plasma. This attractive hypothesis, which would also explain the absence of hypercholesterolemia in the presence of hepatocellular damage (viz., impaired bile acid formation and no increase in plasma bile acids), is based on observations in rats and must be tested in other species before its validity can be established.

Patients with jaundice of extrahepatic obstructive origin not infrequently present normal or even subnormal plasma cholesterol values. Such findings are usually dependent upon the presence of some complicating factor, among the most common of which are cachexia, infection, superimposed hepatocellular damage or terminal cholemia. The occurrence of hypocholesterolemia in these conditions is discussed elsewhere (p. 120 ff.). Under such circumstances, the cholesterol ester fraction is abnormally low and the ratio of esterified to free cholesterol is decreased. When this phenomenon is observed in patients with common duct obstruction it should be regarded as of serious prognostic significance, since it usually indicates the presence of one or more of the complicating factors enumerated above.

Hypercholesterolemia occurs at times in cases of mild hepatocellular jaundice (hepatitis), but much less frequently than in obstructive jaundice. When it does occur, it is usually dependent upon an increase in the free cholesterol fraction, the ester cholesterol-free cholesterol ratio being diminished. Except in the terminal stages, plasma cholesterol concentration and partition are normal in portal cirrhosis and are either normal or insignificantly elevated in cholecystitis or cholelithiasis without biliary obstruction. The hypothesis of a relationship of cause and effect between hypercholesterolemia and cholelithiasis has not been substantiated by recent investigations. Experimental studies indicate that there is no constant relationship between the concentration of cholesterol in the bile and in the blood plasma. When the plasma cholesterol concentration is raised by cholesterol administration in experimental animals, the major portion of the increment is transformed, in the liver, to bile acids and is excreted as such in the bile, and not as cholesterol. It would appear that the formation of gallstones is probably dependent largely upon factors operating within the extrahepatic bile passages.

Hypothyroidism. Untreated hypothyroidism is rather consistently associated with an increase in blood lipids, the degree of hypercholesterolemia being roughly proportional to the diminution in the basal metabolic rate. Values above 600 mg. per 100 cc. have been reported in both adults and children. The increase occurs usually in both ester and free fractions. The plasma phospholipids are also increased. This lipid increment occurs mainly in the β-lipoprotein fraction, and is reflected in an increase in the concentration of S_f 12–20 class of molecules (p. 101). A return to normal usually follows the administration of thyroid extract.

Estimation of the plasma cholesterol concentration may be of value in the diagnosis and regulation of thyroid dosage in the treatment of children with hypothyroidism in whom the determination of the basal metabolic rate may not be practicable. However, although it tends to be elevated, at times markedly (above

500 mg. per 100 cc.), the cholesterol concentration in hypothyroid children has been found to fluctuate within wide limits upon repeated determination and to be within normal limits in some cases of severe hypothyroidism. Decrease in plasma cholesterol during thyroid hormone therapy and a marked increase following cessation of therapy has been found to be of considerable diagnostic significance in children.

Atherosclerosis. The relation of plasma lipoprotein abnormalities to the development or incidence of atherosclerosis is discussed elsewhere (p. 111), as is the basis for interest in the plasma cholesterol concentration in this connection. Despite numerous attempts to demonstrate that hypercholesterolemia plays an important role in the pathogenesis of atherosclerosis, such a relationship has not been established unequivocally. There can be no doubt that the incidence of atherosclerosis is high in certain conditions accompanied by protracted hypercholesterolemia, e.g., diabetes mellitus, nephrosis, hypothyroidism, and idiopathic hyperlipemia. However, its incidence is not unduly high in other conditions, e.g., biliary cirrhosis, exhibiting comparable plasma cholesterol concentrations. Moreover, observations in large numbers of subjects with atherosclerosis have generally failed to reveal any significant consistent abnormality of the plasma cholesterol in the absence of conditions such as those mentioned above. In fact, the current emphasis on studies of lipoprotein fractions has been stimulated largely by the apparent lack of correlation between the level of plasma cholesterol and the incidence of atherosclerosis, attempting to uncover some apparently more significant reflection of altered lipid metabolism, e.g., cholesterol:phospholipid ratio, lipid distribution patterns, etc.

The difficulties involved in attempts to establish such a relationship are indicated elsewhere (p. 113). Studies in experimental animals are very suggestive of a causal relation; e.g., the development of atherosclerosis in rabbits and chicks receiving large amounts of cholesterol, and in dogs in which hypercholesterolemia is induced by thiouracil plus cholesterol feeding. However, the evidence in man is conflicting and confusing. According to some, the mean concentration of plasma cholesterol is somewhat increased in atherosclerotic subjects as compared with normal persons of the same age. This is denied by others. There is more general agreement that the range of variation is considerably greater. Nevertheless, there is little question but that a considerable proportion of subjects with atherosclerosis exhibit no abnormal elevation of plasma cholesterol. This by no means signifies that an abnormality of lipid metabolism or transport is of no diagnostic or pathogenetic significance in this connection, but further studies are required before the possible roles of abnormalities in plasma cholesterol, S_f 12–20 molecules, α- and β-lipoproteins, cholesterol:phospholipid ratio, and chylomicron count (turbidity) can be clarified.

Xanthomatosis. Xanthomatosis is the designation applied to the cholesterol variety of lipoidosis (p. 109). In certain forms of xanthomatosis, i.e., of the so-called primary or essential type, the plasma cholesterol is within normal limits (normocholesterolemic type, p. 110). This category includes the great majority of cases of the Schüller-Christian syndrome. Hypercholesterolemia is present in a small percentage; according to some, it occurs during active stages of the disease, normal values being obtained during periods of apparent quiescence. Hypercholesterolemia occurs also in xanthomatous biliary cirrhosis and in other (hypercholesterolemic, p. 110) types of cutaneous and visceral xanthomatosis.

Xanthomata frequently develop in conditions characterized by protracted hypercholesterolemia, so-called secondary xanthomatosis (p. 110). These conditions include idiopathic hyperlipemia, chronic relapsing pancreatitis, diabetes mellitus, biliary cirrhosis, nephrosis, hypothyroidism, glycogen storage disease, nephrosis, etc. The plasma phospholipids are also increased. With the exception of occasional cases of biliary cirrhosis, the increment occurs largely if not exclusively in the β-lipoprotein fraction.

Idiopathic (Essential) Hyperlipemia. The designation, idiopathic (essential) hereditary hyperlipemia, has been applied to a condition of unknown etiology, with a familial incidence, and a rather characteristic clinical picture of splenomegaly, hepatomegaly, and periodic attacks of acute abdominal pain, during which the previously elevated plasma lipids fall. There is stated to be a nonhereditary form of this condition. There have been reports of total lipid values over 8 gm./100 cc., with cholesterol concentrations over 400 mg. and phospholipids over 450 mg./100 cc. Neutral fat constitutes the bulk of the plasma lipids in this condition, and lipoprotein fractionation shows the increase to be in the beta fraction and in the relatively high S_f molecular classes (p. 111).

Similar attacks of abdominal pains occur in other hyperlipemic states, e.g., diabetes and nephrosis. It has been suggested that they may be due to disseminated fat embolization resulting from alteration in the physical state of the plasma lipids. Other manifestations include transient paralyses, epileptiform seizures, vasomotor collapse, and unexplained gastrointestinal hemorrhage. Certain cases of chronic relapsing pancreatitis, presumably of similar origin, have also been observed to be associated with hyperlipemia, which usually persists in symptom-free periods.

Miscellaneous. Whereas the plasma cholesterol is often low in patients with other forms of anemia (p. 120), high values may be obtained following acute hemorrhage. This increase may be pronounced within forty-eight hours after the acute blood loss, and may persist for several days. The cause is not known. It may be due to anoxia and excessive mobilization of lipids from the fat depots.

Hypercholesterolemia has been observed occasionally in patients with hypertrophic osteoarthritis, senile cataract, psoriasis, and dermatitis. Its occurrence in these conditions is probably fortuitous.

There have been claims of an increase in the plasma phospholipid : cholesterol ratio in eclampsia, due to an independent increase in phospholipids, with no significant change in cholesterol. This may possibly be due to an increase in the concentration of beta- at the expense of alpha-lipoproteins, which has been reported to occur in certain types of kidney disease, e.g., glomerulonephritis, intercapillary glomerulosclerosis (p. 112). The occurrence of hyperlipemia (including cholesterol) in the hepatic type of glycogen storage disease (von Gierke) is referred to elsewhere (p. 57).

HYPOCHOLESTEROLEMIA

Anemia. The plasma cholesterol is uniformly low in pernicious anemia during relapse, and in hemolytic jaundice, values as low as 50 mg. per 100 cc. having been reported. Hypocholesterolemia is likewise observed in practically all patients with severe hypochromic anemia with the exception of severe aplastic anemia and anemia following acute hemorrhage. In severe hypochromic anemias and also in pernicious anemia and in hemolytic jaundice the diminution in plasma cholesterol is associated with reduction in plasma phosphatide and an increase in fat, these changes occurring typically with hemoglobin values below 50 per cent but bearing no direct relation to the degree of anemia. In typical cases of pernicious anemia in relapse the cholesterol rises suddenly at the onset of remission, whether spontaneous or induced by treatment. This occurs simultaneously with the reticulocyte response and occurs usually before any increase in hemoglobin or red blood cells. Consequently, normal or even elevated plasma cholesterol values may be present in patients with severe anemia, provided that remission has begun. This may account for certain of the discrepancies in reported observations of plasma cholesterol concentration in this condition.

Hepatic Disease. Hepatocellular damage is frequently accompanied by a diminished proportion of cholesterol esters in the blood plasma (normally 60–80% of the total), constituting the so-called "Estersturtz." This may not be, but usually is, associated with a diminution in total cholesterol concentration. The chief practical significance of this observation lies in the fact that it offers some hope of differentiating hepatocellular jaundice from simple obstructive jaundice, in which hypercholesterolemia is the rule (pp. 116, 514). Hypocholesterolemia, with a diminished proportion of cholesterol esters, has been observed in cases of hepatocellular damage due to the following causes: (*a*) drugs, such as cinchophen, phenobarbital, chloroform, carbon tetrachloride and phosphorus; (*b*) pneumonia, myocardial failure, yellow fever and spirochetal jaundice; (*c*) infectious (viral) and toxic hepatitis, and acute, subacute and chronic diffuse necrosis of the liver. Similar findings have been obtained in experimental animals.

A pronounced discrepancy between the degree of bilirubinemia and of cholesterolemia is usually observed in degenerative conditions of the liver (hepatocellular damage); the more severe the hepatic damage the greater the tendency toward hypocholesterolemia. This discrepancy between the hyperbilirubinemia and cholesterolemia is of value in differentiating between purely mechanical obstructive lesions and superimposed or primary degenerative lesions of the hepatic parenchyma. In parenchymatous disease of the liver, a drop in the ester fraction often parallels the severity of the hepatic damage, and in rapidly fatal cases this fraction may be low or absent throughout the course of the disease. In the event of improvement, the initially low ester values eventually increase. Low values may also be obtained in terminal stages of portal cirrhosis. When this phenomenon is observed in patients with common duct obstruction it should be regarded as of serious prognostic significance, since it is indicative usually of extensive superimposed hepatocellular damage.

The occurrence of hypocholesterolemia in these conditions is explicable on the basis of present knowledge concerning the role of the liver in the synthesis of the plasma cholesterol. Experimental evidence suggests that the hepatic cell is probably the only important site of formation and esterification of the plasma cholesterol (p. 99). Impairment of liver cell function would therefore be expected to result in a decrease in ester and total cholesterol levels. The usefulness of these determinations in the differential diagnosis of obstructive and hepatocellular jaundice is discussed elsewhere (p. 514).

Infection. Hypocholesterolemia and a diminution in the concentration of other blood lipids is observed frequently during the course of acute infectious diseases. The most marked decrease usually occurs in the ester fraction which, together with the total cholesterol concentration, rises to normal and sometimes supernormal levels during convalescence. Similar changes occur simultaneously in fatty acids and phospholipids. There appears to be no constant relationship between the degree of hypocholesterolemia and the severity of the clinical manifestations. Such changes cannot be produced by the induction of artificial fever. According to some observers, hypocholesterolemia is of prognostic significance in tuberculosis, in which condition it appears to be related to the severity of the process. The cause of these changes in plasma cholesterol in infectious diseases is not understood, but they may be related in some way to the phenomenon of immunity. This is suggested by the observation that the fall in temperature in pneumonia induced by administration of specific serum is followed promptly by increase in cholesterol (and fatty acids) whereas similar fall in temperature and clinical improvement induced by sulfonamide therapy are followed by a significant increase in cholesterol only after an interval of four to seven days. This corresponds approximately to the time at which type-specific antibodies appear in the blood in sulfonamide-treated cases.

Hyperthyroidism. In thyroid disease, there appears to be a roughly reciprocal relationship between basal metabolic rate and plasma cholesterol concentration. Low values (60–100 mg. per 100 cc.) may be obtained in patients in or near thyroid crises. The average values in exophthalmic goiter are somewhat lower than in toxic nodular goiter. The apparent absence of such changes with increased basal metabolic rates due to administration of dinitrophenol suggests that the changes in plasma cholesterol in thyroid disease are not directly related to the metabolic rate but to other actions of thyroid hormone. Diminution in the basal metabolic rate in patients with hyperthyroidism following administration of iodine, antithyroid drugs, or subtotal thyroidectomy is usually accompanied by an increase in plasma cholesterol concentration.

Determination of plasma cholesterol is of little clinical value in hyperthyroidism. The nutritional status exerts a considerable influence and, whereas the plasma lipid level may be related to the degree of thyroid activity in uncomplicated cases, the problems of diagnosis and prognosis in such cases are relatively simple and are not usually further clarified by investigation of the plasma cholesterol concentration. Moreover, the latter has been found to be no criterion of the probable response of the patient to thyroidectomy or other specific therapy.

Inanition. A diminution in plasma cholesterol is commonly observed in conditions accompanied by wasting and cachexia. Its significance in these conditions is not clearly understood. In many cases there is an associated decrease in plasma protein concentration, both of these phenomena apparently being directly related to the state of malnutrition.

Terminal States. The frequent occurrence of hypercholesterolemia in the nephrotic syndrome has been referred to elsewhere (p. 115). In non-nephrotic forms of glomerulonephritis, however, although the total fat, fatty acid and phospholipid concentrations in the plasma are commonly increased, plasma cholesterol is frequently normal or subnormal, this dissociation suggesting a functional differentiation between these lipid fractions. The variability of the plasma cholesterol in chronic glomerulonephritis has attracted considerable attention, practically all investigators of this problem coinciding in the opinion that a fall from a previously elevated or normal level is of serious prognostic significance, particularly if associated with increasing nitrogen retention. There usually appears to be a roughly reciprocal relationship between these two factors in the terminal stages of this disease, although there is no constant quantitative relationship between the degree of cholesterolemia and of nitrogen retention.

Low plasma cholesterol values (50–100 mg. per 100 cc.) have also been obtained in patients in the terminal stages of a variety of diseases without associated nitrogen retention. Among these are arteriosclerosis, congestive heart failure, carcinoma, acute pancreatitis, pulmonary tuberculosis, bacterial endocarditis and other forms of bacteremia, coronary artery occlusion and diabetes mellitus.

No definite statement can be made at the present time regarding the cause of hypocholesterolemia in terminal states. Some believe that it is due to anemia and cachexia, which are frequently present in such patients. However, these factors are frequently absent in those dying of peritonitis, pneumonia, coronary artery occlusion, congestive and left sided heart failure and in certain patients with urinary obstruction due to prostatic enlargement. If one is to assume that the mechanism underlying the production of hypocholesterolemia in various terminal states is essentially the same in each case, it would appear that some other explanation must be sought. There is some evidence that this mechanism involves an increased rate of removal of cholesterol from the blood as a result of a state of increased reticulo-endothelial cell activity. During periods of stress, both physical and mental, the adrenal cortex is stimulated to increased activity, an important feature of the "alarm reaction" or adaptation syndrome. As indicated elsewhere, such stimulation is accompanied by a decrease in adrenal cholesterol (p. 426), continuing to marked depletion in severe conditions of stress. It is possible that under such circumstances, the cholesterol stores of the body may be depleted, leading to hypocholesterolemia. A similar mechanism may operate in severe infections (p. 121). It should be recalled, however, that experimental evidence indicates that, under physiological conditions at least, very little cholesterol is removed from the plasma by tissues other than the liver. Whatever may be the cause, the development of hypocholesterolemia under such circumstances is of serious prognostic significance, especially in diabetes mellitus and chronic glomerulonephritis, in which the plasma cholesterol may previously have been elevated.

Miscellaneous. Hypocholesterolemia has been observed in patients with prostatic obstruction and with intestinal obstruction, its occurrence in these conditions being of rather serious prognostic import and indicative of poor defensive powers. Low plasma cholesterol values have also been reported during epileptic seizures and in arthritis.

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CHAPTER III

Protein Metabolism

Proteins may be defined as compounds of high molecular weight, consisting largely (or entirely) of chains of α-amino acids united in peptide linkage. The constituent amino acids can be obtained by hydrolysis of the proteins. While the individual amino acids may be considered analogous to the monosaccharides of carbohydrate chemistry, and the true proteins analogous to the polysaccharides, an important difference must be pointed out. The polysaccharides are polymers of a single sub-unit, or of a few types of sub-units at the most, while the proteins contain, in general, some twenty-odd individual amino acids, present in characteristic proportions and linked in a specific sequence in each protein. Hence, other things being equal, it is possible for nature to concoct many more different proteins than polysaccharides, a fact which lends fascination as well as difficulty to the problems confronting the protein chemist.

The molecular weights of the proteins vary from about 13,000 to many millions. Most proteins, owing to their molecular size, are not diffusible through membranes such as cellophane and, like the polysaccharides, are actually of colloidal dimensions and exhibit the properties associated with the colloidal state of matter. Some are soluble in pure water; some require the presence of salts or small amounts of acid or base to dissolve. One group is soluble in certain concentrations of alcohol, although (with the exception of the recently discovered proteolipids) proteins are generally insoluble in organic solvents. The structural proteins known as scleroproteins are dissolved only by reagents which cause considerable alterations in their structure.

The average protein is a very sensitive individual. In reprisal for its exposure to heat, extremes of pH, surface action, or various reagents, the native protein undergoes a series of changes known as denaturation, resulting in alterations in a number of its properties. Heat denaturation, under the proper circumstances, proceeds to a further state, coagulation.

The biochemical significance of proteins cannot be overemphasized. If carbohydrates and lipids, generally speaking, can be considered the fuels of the metabolic furnace, proteins may be regarded as forming not only the structural framework, but also the gears and levers of the operating machinery. Indeed, at the risk of pushing the analogy to extremes, we may regard the protein hormones (which acts as regulators of metabolism) as the policy-forming top management of the enterprise.

Such structures as cell walls and various membranes are protein in the main, although frequently coupled or associated with lipids. Connective tissue is composed of chemically rather inert types of proteins, in some cases combined with carbohydrates. The contractile elements of muscle are now thought to be proteins. Aside from such obviously structural substances as those mentioned, the catalysts which permit the reactions of intermediary metabolism to proceed at a reasonable rate under mild conditions, the enzymes, are known to be protein in nature. Finally, as already pointed out, certain proteins in the body act as hormones, or regulators of metabolism.

Protoplasm may be regarded as a colloidal system of proteins, together with some lipids and carbohydrates. With some exceptions, the supply of carbohydrates and lipids may rise or fall, and the cell may flourish or do poorly as a result, but at any rate it survives for some time. If something interferes with the supply of protein, however, the cell inevitably sickens and dies.

DIGESTION AND ABSORPTION

Ingested proteins undergo digestion in the stomach and small intestine. This process consists essentially in hydrolysis into their constituent amino acids, with proteoses, peptones and polypeptides as intermediary products.

The factors in gastric juice important in this connection are (1) hydrochloric acid and (2) pepsin, a proteolytic enzyme. The functions performed by HCl are: (*a*) conversion of protein to acid metaprotein; (*b*) activation of pepsinogen to pepsin; (*c*) provision of an optimum pH for peptic activity (about 1.5 to 2.2, varying somewhat for different proteins). Under optimum conditions in vitro, pepsin is capable of splitting proteins to the stage of amino acids, but under normal conditions of gastric emptying (p. 471), there is very little digestion beyond the state of peptones, 45 to 70 per cent of the ingested protein being in the form of proteose at the time it leaves the stomach.

Rennin is a milk-curdling enzyme which acts upon the casein of milk, forming a proteose- or peptone-like body and soluble paracasein. The latter is converted to insoluble calcium paracasein (curd), which undergoes peptic digestion. It has little or no protein-digesting activity and is apparently not present in the gastric juice of adult humans, in whom pepsin serves as a milk-curdling enzyme. Rennin occurs abundantly in the gastric juice of the calf and other ruminants, and probably also in the human infant, in whom pepsin is present in comparatively small amounts.

Digestion of protein and protein products in the intestine is continued by proteolytic enzymes of the pancreatic and intestinal secretions. Pancreatic juice contains trypsinogen, chymotrypsinogen and carboxypolypeptidase, the first two being activated by enterokinase, an enzyme secreted by the intestine. Carboxypolypeptidase breaks down polypeptides to simpler peptides and amino acids, whereas the other two enzymes act on native proteins to form proteoses, peptones, polypeptides, simpler peptides and amino acids, depending upon the length of time the reaction is allowed to proceed. In addition to enterokinase, the intestinal juice contains dipeptidase and aminopolypeptidase (formerly jointly termed erepsin), which split polypeptides to amino acids.

Nucleoproteins are split by proteolytic enzymes into protein and nucleic acids. The protein thus formed undergoes digestion in the stomach and intestine as outlined above. The nucleic acid is converted, by the action of nucleases in the lumen and wall of the intestine, to nucleotides (purine and pyrimidine), which are hydrolyzed by phosphatase in the intestine into phosphoric acid and the nucleosides, adenosine and guanosine, and pyrimidine substances.

The process of protein digestion consists essentially in the production of progressively smaller peptides by cleavage of internal peptide linkages (endopeptidases) and liberation of amino acids by cleavage of terminal peptide linkages (endo- and exopeptidases). If an intact "foreign" protein, i.e., other than human, is introduced into the organism, it acts as an antigen, i.e., it stimulates the formation of specific antibodies, "sensitizing" the organism to this protein (allergic state). Subsequent exposure to the latter results in a serious, and sometimes fatal reaction (anaphylaxis). Obviously, if this phenomenon is to be avoided, food proteins must be so altered before absorption that they have lost their species specificity and thereby their capacity for inducing this state of sensitization. This is accomplished effectively by digestion to the stage of amino acids, which, of course, exhibit no such specificity. It is possible, too, that small peptide molecules escape complete hydrolysis and are absorbed without harmful effect. There is immunological evidence, however, that intact native protein molecules may occasionally be absorbed from the intestine in an unaltered state, constituting the basis for allergic reactions in certain cases.

Food proteins are generally readily digested (90–97 per cent) under normal conditions, very little escaping in the feces. The only important exception is the insoluble fibrous protein, keratin, which is not hydrolyzed by enzymes of the human digestive tract. Most proteins are profoundly altered by many procedures commonly used in the preparation of foods. Heating to coagulation temperatures causes polymerization; superheated steam, excluding air (pressure cookers), causes hydrolysis; dry heat may cause oxidation. In the majority of instances, the digestibility and biological value (p. 219) of these proteins are not affected by such procedures. However, proper cooking may facilitate digestion and utilization, e.g., cooked egg albumin is digested more readily than raw; heating soybeans increases their biological value by inactivating a component with antitryptic activity. The nutritional value of cereal proteins is lowered by overheating or toasting (e.g., certain breakfast cereals).

With the few exceptions indicated above (i.e., small peptides, occasionally native protein), food proteins enter the organism in the form of their constituent amino acids. Certain other aspects of protein digestion may have an important bearing on their nutritional value, e.g., variation in the rates of liberation of amino acids. The amino acids are readily soluble in water and are promptly absorbed from the small intestine, mainly into the portal circulation (to the liver), and to a much smaller extent, via the lacteals, into the thoracic duct and thence directly into the systemic circulation. Only small amounts of free amino acid are found in the intestinal contents during the process of digestion, indicating the rapidity of their absorption. This is reflected also in the rather prompt postprandial increase in blood amino acid concentration (p. 136).

Dynamic State. It was realized even for some time prior to the use of isotopes, but particularly clearly since the introduction of labeling technics, that the constituents of the body are in a constant state of flux. All body proteins, including plasma proteins, hemoglobin, and intracellular proteins, continually undergo degradation and synthesis, more than half of the protein of the liver and intestinal mucosa being broken down and resynthesized in ten days; the turnover is slower in muscles and erythrocytes. Active resynthesis occurs even during periods of starvation, and active breakdown during periods of nitrogen equilibrium; degradation of protein in one tissue may be accompanied by synthesis in others. Antibody proteins (γ-globulins), induced by active immunization, also undergo continual breakdown and synthesis, the half-life of these and other plasma proteins being about two weeks.

Protein in one compartment of the organism may be drawn upon to supply a deficiency in another compartment. For example, either plasma protein or hemoglobin (in the dog), given intravenously, may supply the protein requirements during prolonged fasting periods.

The apparent stability of the adult organism is the result of a balance between the rates of synthesis and degradation of its constituents. In a growing organism, the rates of synthesis of many of its constituents must exceed the rates of breakdown, in order that new tissue may be constructed. Wasting diseases, starvation, and related states are characterized by rates of catabolism which are greater than the rates of anabolism.

In a given physiological (or pathological) state, the rate of synthesis or degradation of a specific compound will be characteristic of (1) the chemical nature of the compound in question, (2) its location in a particular tissue or organ, and (3) its intracellular location.

Several methods of expression are currently used to describe the rate of synthesis or breakdown of a body constituent. Most of these have been developed in connection with isotopic-labeling technics. The "half-life" is the time required for replacement of one-half of the molecules of the compound in question, the definition being analogous to that used in describing the unstable isotopes. The "average life" of a given compound is the time required to replace the molecule of "average stability." Sometimes the rate of a synthetic or degradative process is expressed directly in terms of mass of compound transformed per unit time per unit weight of tissue or animal, e.g., "mols of urea synthesized per day per kilo body weight." On other occasions, the rate of turnover of a compound is stated as the percentage of the amount present which is metabolized per unit time.

Metabolic Pool. Since the "endogenous" molecules are in a state of flux, they and their metabolites must be constantly mixing with those derived from the diet ("exogenous"). This mixture of originally endogenous and exogenous materials, which is drawn upon for both anabolic and catabolic reactions, constitutes a reservoir or "metabolic pool" of the compounds in question. For example, the alanine derived from the continuous breakdown of body protein and that obtained from dietary protein mix to form an "alanine pool." The amino group of the alanine, together with the amino groups of all other amino acids which are able to participate in nitrogen exchange reactions (e.g., transamination), form a pool of metabolically labile nitrogen. This pool is contributed to by substances derived from catabolic processes in the tissues and substances absorbed from the intestine. Catabolism of proteins is stimulated by 11-oxygenated adrenocortical hormones, and excessive amounts of thyroid hormone. Protein anabolism is stimulated by pituitary growth hormone, insulin, testosterone, and, at least in the growing organism, by physiological amounts of thyroid hormone.

Through certain compounds such as pyruvate, acetate, oxaloacetate, α-ketoglutarate, etc. (pp. 13, 138), the metabolism of protein is integrated with that of carbohydrate and fat, members of one pool being thus metabolically equilibrated with other pools. Alanine, for example, is reversibly converted by deamination to pyruvic acid; hence the carbon skeleton of this amino acid is involved in the pyruvate pool, which is directly connected to the carbohydrate pool.

The size of a pool is the quantity of constituent instantaneously present and available for all of the reactions leading into and from that particular pool. Although the quantitative determination of "pool size" with the aid of isotopes has been begun only recently, a few data are already available. The pool of metabolically labile amino groups in the human being, for example, has been estimated at 2 gm. of nitrogen (70-kg. man), i.e., of the same order of magnitude as the free amino acids of the tissues.

The concepts of dynamic state and metabolic pool have been invaluable in the interpretation of data resulting from experiments with isotopic labeling. For example, consider the case of an adult experimental animal in nitrogen balance being fed a dose of glycine labeled with N^{15}. According to the classical theories of biochemistry, the metabolic paths of endogenous and exogenous compounds are compartmentalized; hence exogenous glycine, since it is not required for tissue construction (adult animal, nitrogen equilibrium), should be degraded directly and the nitrogen excreted as urea within approximately twenty-four hours. What is found experimentally is that a quantity of urea is excreted equivalent to the glycine administered. However, only a small fraction of the N^{15} is excreted in this urea; several days are required for the recovery of an appreciable fraction. Hence, the nitrogen output is equivalent to the intake, but the atoms excreted are mainly not those ingested. To begin with, the ingested glycine enters the glycine pool, so that the concentration of N^{15} in the average glycine molecule is lowered by dilution with all of the N^{14}-glycine molecules. Secondly, the amino group of glycine is readily equilibrated with that of most other amino acids (transamination p. 135), resulting in further dilution or spread of N^{15} throughout most of the amino acids of the body. The nitrogen pool which is drawn upon for the synthesis of urea, therefore, contains a much lower concentration of molecules labeled with N^{15} than did the sample of glycine administered. It is obvious that the classic concept of independent exogenous and endogenous metabolism is directly contradicted by such observations, and can no longer be maintained.

OVER-ALL METABOLISM OF PROTEIN

Much of the information on the metabolism of protein can be expressed (and is frequently measured) in terms of nitrogen, the reasons for which are readily apparent. The carbon and hydrogen atoms of the proteins form CO_2 and H_2O as end-products of metabolism, indistinguishable from the end-products of the metabolism of carbohydrates or fatty acids. Although certain proteins contain phosphoric acid, this constituent is more characteristic of the nucleic acids, phospholipids, and certain intermediates in carbohydrate metabolism. Sulfur is an almost invariable constituent of proteins, but its metabolism reflects only the reactions of cystine and methionine. In contrast to these elements, nitrogen is uniquely characteristic of proteins, from the standpoints of both its high concentration in the protein molecule (average, 16%) and its specialized excretory products (ammonia, urea, etc.)

Nitrogen of the Food. Protein nitrogen outweighs all other forms of nitrogen in the diet. Traces of inorganic nitrogen are ingested in the form of nitrates and nitrites. Small amounts of organic nonprotein nitrogen (NPN) are also present in the food, including nucleic acids and their derivatives, and amino acids and peptides.

Nitrogen of the Body. Many nitrogenous compounds are found in the tissues and fluids of the body. Protein itself averages about 20 per cent of the wet weight of most tissues. In the blood, in addition to protein, several types of NPN are found. Urea, the major waste product of protein catabolism, formed in the liver, passes into the blood stream to the kidneys for excretion. Blood creatine may be en route to muscle for the synthesis of phosphocreatine, or may have leaked out of muscle. Creatinine is a waste product, formed from phosphocreatine and creatine. Uric acid is the end-product of purine catabolism. Free amino acids are also found in the blood; they are in transit from one organ to another, for purposes of synthesis or breakdown. Other components of the blood NPN include polypeptides, glutathione, purines and pyrimidines, ATP, and ergothionine.

Excretion of Nitrogen. Fecal nitrogen appears not to be related to the nitrogen of the ingested protein. Its quantity varies with the bulk of the diet, and does not normally represent unabsorbed dietary protein. In the adult human, it amounts to 1 to 2 gm. N/day. Perspiration, unless sweating is excessive, accounts for a loss of only 0.3 gm. N/day or less.

The urine is the major route of excretion of nitrogen (p. 149). In the average, normal, human adult, the total nitrogen of the urine is about 13 gm./day, and this is normally all NPN (no significant quantities of protein are found in the urine normally). Urea (ca. 85% of the total) and ammonia (ca. 3%) vary directly with the level of protein in the diet. Creatinine (5% of the total) excretion is related to the muscle mass and is quite constant for an individual. Creatine is not normally excreted by adult males or by most females. Only traces of amino acids are found in the urine. Uric acid (1% of total) output fluctuates with the level of dietary purines. About 5 per cent of the total is composed of compounds not ordinarily determined. The factors which influence the urinary excretion of the various nitrogenous substances are discussed elsewhere (p. 149ff.).

Nitrogen Balance. Since most of the nitrogen of the diet represents protein, and most of the nitrogenous excretory products are derived from protein catabolism, it is apparent that the balance between the two will reveal significant features of protein metabolism. Nitrogen balance is defined as the quantitative difference between the nitrogen intake and the nitrogen output, both expressed in the same units (such as grams N/day). By intake is meant the nitrogen of the food. Included in output are such routes of excretion as urine, feces, milk, sweat, expectoration, vomitus, desquamation of skin, menstrual fluid, and loss of hair; in practice, however, only the first two are taken into account, except under unusual circumstances.

Positive nitrogen balance exists when intake exceeds output. This condition obtains whenever new tissue is being synthesized, such as during growth of the young, in pregnancy, and in convalescence from states of negative nitrogen balance.

In negative nitrogen balance, the output exceeds the intake. This condition, which obviously cannot continue indefinitely, occurs on inadequate intake of protein (fasting, diseases of the gastrointestinal tract), in states of accelerated catabolism of tissue protein (fevers, infections, wasting diseases), and when the loss of protein from the body is accelerated in some way (lactation on inadequate diets, albuminuria). Negative nitrogen balance occurs also in the absence from the diet of an "essential" amino acid.

From the preceding discussion, it is evident that nitrogen equilibrium (intake = output) can be achieved only in the adult organism, and only in the absence of the abnormal conditions enumerated above. An "adequate diet" must also be specified, which means that all requirements for minerals and vitamins are fulfilled, that the protein of the diet is of high "biological value" and is administered at a sufficiently high level, and that the caloric needs of the body are met satisfactorily by the carbohydrate and fat of the diet. A certain minimum amount of dietary carbohydrate is required for the maintenance of nitrogen equilibrium (5 gm./100 Calories), independently of the adequacy of the energy provision from fat and protein. This is referred to as the "protein-sparing action" of carbohydrate (pp. 133, 216). In these circumstances, the nitrogen output equals the intake, and an increase or decrease in the intake is followed, within a day or two, by a corresponding adjustment in the output, so that nitrogen equilibrium is established at a new level. There appears to be no marked tendency toward storage of surplus nitrogen, as there is in the case of the fats and carbohydrates.

Essential Amino Acids. It is possible to replace the protein of the diet completely with pure amino acids. The so-called "dietary requirement" for protein is, therefore, really a requirement for amino acids, and is expressed in terms of protein only because the amino acids of the available foodstuffs are found in that form.

By the elimination of single amino acids from an otherwise complete diet, it has been found that the organism can successfully dispense with certain of these compounds, but not with others. Evidently all amino acids are not synthesized with equal facility by the body. An "essential," or "indispensable," amino acid is defined as one which cannot be synthesized by the organism, from substances ordinarily present in the diet, at a rate commensurate with certain physiological requirements. The definition includes the phrase, "cannot be synthesized by the organism, from substances ordinarily present in the diet . . . ,'' because certain of these amino acids may be replaced by the corresponding α-keto or α-hydroxy acids. The requirements specified in the most extensive series of investigations are: (1) optimal growth of the young, and (2) maintenance of nitrogen equilibrium in the adult.

Although the amino acid requirement for growth has been investigated in several species, the most thorough experiments have been performed in the white rat. The ten amino acids required by this animal for optimal growth (and, in the absence of data to the contrary, the same list is assumed to apply to other mammals, including man) are: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, arginine, and histidine. In the case of arginine, the requirement is not absolute; the animals grow, but sub-optimally, in its absence, indicating a slow and (for this physiological requirement) inadequate rate of synthesis of this amino acid.

The requirement for nitrogen equilibrium in the adult appears to be less stringent. In the human being (more specifically in graduate students at the University of Illinois) only eight amino acids are required for maintenance of nitrogen equilibrium, the list differing from the preceding in the absence of histidine and arginine.

A curious fact concerning the essential amino acids is that the complete group must be administered to the organism at the same time. If a single essential amino acid is omitted from the group and fed separately several hours later, the nutritional effectiveness of the entire group is impaired. From this fact, and from the effects on growth and nitrogen balance of the omission of a single essential amino acid from the diet, it seems probable that the organism will synthesize either complete proteins or none at all, and that the entire fabric of the protein is formed, if not simultaneously, within a relatively short period of time.

The lists of essential amino acids must be taken provisionally, particularly in their application to human nutrition. In the case of the ten amino acids required for growth (rat), there is a certain risk in transferring nutritional requirements from one species to another. Although the nitrogen equilibrium experiments in human adults seem satisfactory, they were run for periods of time which represent only small fractions of the lifetime of the individual. It is possible that additional requirements, quantitative and qualitative, exist if longer periods of time are considered, or if the individual is subjected to stress.

It has been claimed that proteins contribute something to nutrition in addition to amino acids, and that experimental animals grow more rapidly on diets of protein than on mixtures of amino acids simulating the composition of the protein. The difference is attributed to small peptides resulting from incomplete digestion of the protein. One such peptide (of unknown structure) has been called ''strepogenin,'' since it appears to stimulate the growth of certain streptococci. This subject is controversial at present, and it is by no means certain that the effects attributed to peptides derived from partially hydrolyzed proteins are not due to the association of certain B vitamins with the proteins in natural foods.

Biological Value of Proteins. In order that a food protein may have value to the organism, it must be digested and absorbed. Keratins, for example, are practically refractory to the action of digestive enzymes, and consequently are of no value in the diet. Even if digestible, a protein may be nutritionally inadequate owing to the absence (or presence in inadequate amount) of an essential amino acid. Gelatin (no tryptophan) and zein (no lysine, low tryptophan) are in this category. In general, animal proteins are nutritionally superior to plant proteins. The latter may be satisfactory if the food contains a sufficiently wide variety, but many individual plant proteins are seriously deficient in one or more of the essential amino acids.

Dietary Protein Requirements. (*a*) General Nutrition. If it is assumed that the protein of the diet is biologically adequate, the nutritional requirements can be discussed in terms of quantities of protein, rather than of amino acids. As a further assumption, sufficient fat and carbohydrate must be included in the diet to cover the caloric requirements of the individual. Increased requirements occur in certain situations, viz., pregnancy, lactation, and convalescence.

In connection with protein nutrition, the subject of "storage protein" is of interest. From the relatively rapid adjustment of nitrogen excretion to variations in intake in adult organisms, it appears that no significant quantity of protein is stored. It is nevertheless true that, in states of protein deprivation, certain tissues are able to contribute significant quantities of their protein for the preservation of certain vital functions. Although there is no chemically or morphologically distinct storage form of protein, corresponding to glycogen or depot fat, the more metabolically labile proteins of the tissues are available in case of need. These include the plasma proteins (however, other tissue proteins are usually sacrificed to provide plasma protein), and the proteins of the liver, gastrointestinal tract, and kidney. Only a small percentage of muscle protein appears to be labile, but the size of the muscle mass is such that it provides the greatest absolute amount of protein in conditions of deprivation.

(*b*) Synthesis of Special Proteins. Owing to practical considerations, studies of nutrition of necessity have been confined largely to the entire animal, although it is obvious that the nutritional requirements of the organism are a composite of the requirements of the individual tissues, complicated somewhat by tissue-tissue interactions.

If some of the blood is removed from an animal, centrifuged, and the cells suspended in saline and reinjected into the same animal, it is possible to lower the plasma protein level considerably ("plasmapheresis" technic). Initial bleedings of this type in dogs result in rapid regeneration of the plasma protein (from the labile protein of the tissues). Repeated plasmapheresis of dogs kept on appropriate diets eventually depresses the plasma proteins to a low, constant level. The effect can then be tested of various proteins or amino acids in the diet on the synthesis of plasma proteins. In order of decreasing potency in influencing regeneration of plasma protein are the proteins of serum, liver, egg-white, and milk. Among the amino acids, cystine and tyrosine are particularly important.

Similar experiments in which the red cells are removed and the plasma replaced lead to an anemia-type of depletion. Iron, of course, is very important in the regeneration of hemoglobin. Plasma proteins, if fed, are quite effective in hemoglobin synthesis, as are liver, egg white, and kidney (order of decreasing potency). It is of interest that, in animals suffering from a double deficiency (plasma proteins and hemoglobin), the process of regeneration favors hemoglobin over the plasma proteins. Recent experiments have shown that certain proteins preferentially promote the regeneration of one fraction of the plasma proteins, e.g., lactalbumin appears to stimulate the synthesis of plasma albumin, but has little effect on globulins.

As might be expected, dietary proteins effective in the repletion of the protein of one depleted tissue often are ineffective in the "nutrition" of another. Restoration of the protein content of the livers of animals which have been deprived of proteins for various periods of time is accomplished effectively by certain dietary proteins which are considerably less efficient in the regeneration of plasma proteins.

(*c*) PROTEIN-SPARING EFFECTS. It has been observed that the dietary requirement for protein is influenced markedly by the level in the diet of fat and carbohydrate, these latter foodstuffs appearing to have a "protein-sparing" effect. This phenomenon is best understood by consideration of the physiological functions of the major foodstuffs. Fat, although it has certain structural uses, functions primarily as a fuel (9 Cal./gram). Carbohydrate also serves as a fuel (4 Cal./gram), but in addition is required for the synthesis of certain catalytic compounds of metabolic cycles (e.g., oxalacetic acid in the tricarboxylic acid cycle), and provides the carbon skeletons for the synthesis of the non-essential amino acids. Regardless of the adequacy of caloric intake in the form of proteins and fat, at least 5 gm. of carbohydrate per 100 Calories must be supplied if nitrogen equilibrium is to be maintained (p. 216). Protein has an obvious structural role, but even more important from the dynamic point of view is its catalytic function, the enzyme proteins forming a major part of the actual working machinery of the body.

In the absence of fat and carbohydrate from the diet, protein must be degraded to provide fuel (4 Cal./gram) and catalytic compounds of metabolic cycles. Essential amino acids may be broken down to supply the raw materials for the synthesis of the non-essential amino acids. To provide for these increased burdens, the protein intake consequently must be increased. Conversely, addition of fat to the diet will take care of the caloric needs, a relatively small amount of carbohydrate will furnish the catalytic compounds of the cycles and carbon skeletons of the non-essential amino acids, and the protein requirement will be decreased as a result.

(*d*) PROTEIN DEPRIVATION. As stated previously, protein forms the structural framework of the body and the active machinery as well. Liver, intestinal mucosa, and kidney are sites of very active protein metabolism. The turnover rate of protein is very high in these tissues, and they also happen to discharge functions in protein digestion or metabolism which are important to the body as a whole. Protein deprivation, therefore, is particularly harmful to the activity of these tissues. Impairment of their metabolic efficiency results in further disturbances in protein digestion and metabolism, thus aggravating the state of deprivation. A "cycle of degeneration" sets in, reversal of which becomes increasingly difficult. It is obvious, therefore, why so much emphasis has been placed in recent years on the state of protein nutrition of the patient.

INTERMEDIARY METABOLISM

Protein Turnover. As indicated elsewhere (p. 127), all proteins in the body are constantly breaking down to their constituent amino acids and being resynthesized (dynamic state), the rate of this turnover varying for different proteins in different tissues. The amino acids from the body proteins and those derived from the food form a metabolic pool, the members of which are drawn upon for reactions in either the anabolic or catabolic direction.

The metabolic lability of the proteins in the various tissues can be investigated by noting the rapidity with which each tissue loses protein when the organism is depleted (i.e., on a protein-free diet) and regains protein on repletion. A more recent technic measures the rate of uptake into the proteins of each tissue of an isotopically labeled amino acid, or, in certain cases, the rate of loss of an isotope already incorporated. Whatever the method, high rates of turnover have been shown in plasma proteins, intestinal mucosa, pancreas, liver, and kidney, and low rates in muscle, skin and brain. In man, the half-life of the plasma and liver proteins has been found to be about 10 days, that of muscle about 180 days. Tissue proteins are degraded, presumably by intracellular proteolytic enzymes ("cathepsins"), the intracellular and extracellular amino acids tending to become equilibrated in a common pool, although absolute uniformity of distribution obviously cannot be attained because of the differences in protein composition of various tissues. The concentration of free amino acids within the cells is many times that in the blood plasma.

General Pathways of Protein Metabolism. The most important of the general metabolic pathways of amino acids are illustrated in Figure 16. Some follow special metabolic routes; among these are reactions leading to the final excretion of sulfate (from methionine and cystine) and creatinine (from glycine and arginine). General features of the intermediary metabolism of amino acids of most importance from the clinical standpoint will be outlined here. Further details concerning important metabolites in the blood and urine are discussed elsewhere (pp. 148, 149).

CIRCULATING AMINO ACIDS (p. 148). Amino acids absorbed from the intestine and escaping primary changes in the liver, and those produced by proteolysis in the tissues and not reutilized in situ for synthetic purposes, pass into the blood plasma and are distributed throughout the body.

URINARY EXCRETION. Comparatively little free amino acid is excreted in the urine (p. 151) under normal conditions. These substances pass freely into the glomerular filtrate, but are very efficiently reabsorbed by the renal tubular epithelium. Even when the plasma level is raised by intravenous injection of amino acid mixtures, usually not more than 5 per cent of the quantity administered escapes in the urine.

Synthesis of Nitrogenous Substances. The amino acid pool is drawn upon for formation (i.e., replacement) of a large number of essential substances: e.g., enzymes; other cellular proteins; plasma proteins; hemoglobin; purines and pyrimidines (i.e., nucleotides and nucleic acids); certain hormones (e.g., anterior pituitary hormones, insulin, parathyroid hormone, thyroxine, epinephrine); glutathione; creatine.

Transamination and Deamination. With few exceptions, catabolism of amino acids begins with separation of the amino (NH_2) group from the carbon skeleton, which then becomes an α-keto acid. These reactions occur mainly in the liver. For example, alanine → pyruvic acid + ammonia; glutamic acid → α-ketoglutaric acid + ammonia; aspartic acid → oxalacetic acid + ammonia. These reactions are reversible, and, consequently, amino groups of two amino acids may be exchanged, or an amino group may be transferred from an amino acid to an α-keto acid derived, for example, from the metabolism of carbohydrate, e.g., pyruvic, oxalacetic, or α-ketoglutaric acids, with the formation of new amino acids. Pyridoxal phosphate, a pyridoxine derivative (vitamin B_6), is the coenzyme for transamination reactions. These interactions constitute an important mechanism for the integration of protein, carbohydrate, and fat metabolism (p. 138).

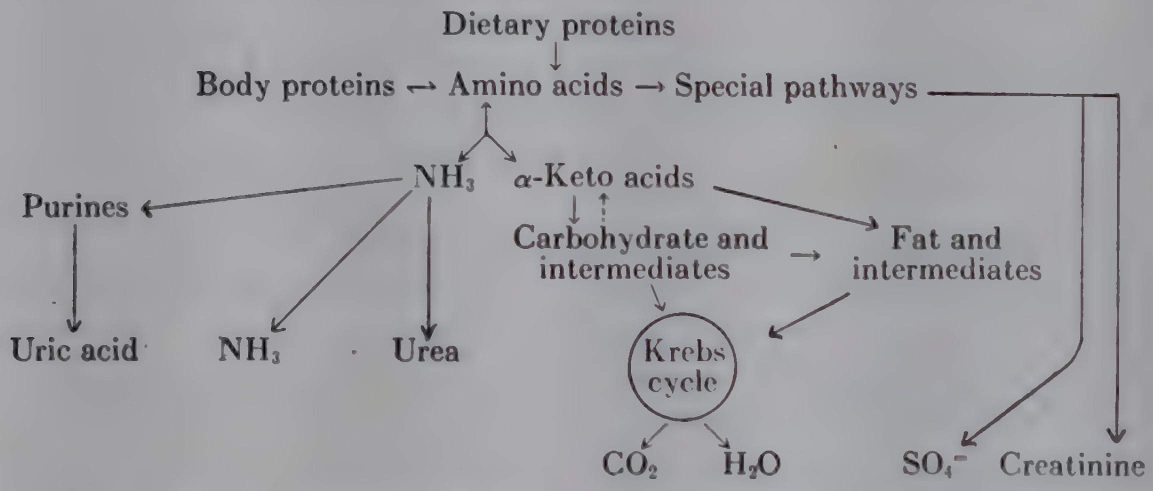

Fig. 16. Metabolic pathways of proteins and amino acids.

Disposal of the Nitrogen. (*a*) *Synthesis.* Ammonia liberated from amino acids may be utilized, as indicated above, for amination of α-keto acids, to form new amino acids. It also participates in the synthesis of purines and pyrimidines (i.e., nucleotides and nucleic acids).

Ammonia is a toxic substance, and large concentrations do not accumulate in cells or extracellular fluids. One of the apparently important mechanisms of its "detoxication" consists in the synthesis of glutamine, an amide of glutamic acid. This compound can thus serve as a carrier of ammonia, which can subsequently be liberated from it by the hydrolytic action of the enzyme, glutaminase.

Certain amino acids enter into the synthesis of other nitrogen-containing compounds without undergoing preliminary deamination: e.g., glycine, in formation of porphyrins (hemoglobin), glutathione, creatine, glycocholic acid;

serine, in formation of choline; arginine, in formation of creatine; cystine, in formation of taurocholic acid; tyrosine, in formation of thyroxine and epinephrine; tryptophan, in formation of nicotinic acid.

(*b*) *Urinary Ammonia.* In the cells of the distal portion of the uriniferous tubules, ammonia is liberated from glutamine (60% of total urine NH_3) and other amino acids (40%) and passes into the lumen of the tubules. This phenomenon, the extent of which is determined, under normal conditions, by the plasma pH, plays an important role in Na conservation by the kidney and, therefore, in the regulation of acid-base balance (p. 331).

(*c*) *Urea Formation.* When amino acids are provided to the organism in excess of requirements for synthesis of new protein molecules and other nitrogenous substances, the excess nitrogen enters into the formation of urea, which is the chief end-product of amino acid metabolism. This occurs only in the liver. NH_3, derived from amino acids by deamination, and CO_2, arising from oxidations (Krebs cycle), are carried by a derivative of glutamic acid (carbamylglutamic acid) and are transferred to another amino acid, ornithine. This undergoes amination (transfer of NH_2 from aspartic acid) to form arginine, which is split by the enzyme, arginase, into urea and a molecule of ornithine, which can then begin again the so-called ornithine-citrulline-arginine cycle. The urea enters the systemic circulation and is excreted from the body, mainly in the urine (p. 149).

In the normal animal, an increase in dietary protein is followed by an increase in the concentration of amino acids in the blood (which are taken up largely by the liver), and by an increase in urinary urea. When liver function is severely impaired, e.g., in acute hepatic necrosis, there is a rise in the blood amino acids and a fall in the urea concentration of blood and urine. Similarly, removal of the liver in an experimental animal is followed by a rise in the concentration of amino acids and ammonia in the blood, and a fall in blood and urinary urea. If the kidneys are removed and the liver left intact, the blood urea rises. Removal of both liver and kidneys results in a constant level of blood urea. From all of these data, it may be concluded that the liver is the chief, if not the sole site of urea synthesis, the precursors of which are amino acids and ammonia. These conclusions are confirmed by perfusion experiments in which it is found that the liver, and no other organ, converts ammonia and amino acids to urea. The bulk of the amino acids (NH_2 groups) not utilized in the organism is thus transformed to urea, which is excreted in the urine as a terminal (waste) metabolite.

DISPOSAL OF THE NONNITROGENOUS RESIDUE. The α-keto acids resulting from deamination may be reaminated, as indicated above, re-forming the original amino acids. Certain of the carbon skeletons are used for special synthetic reactions. The remainder, not required for these replacement purposes, take the pathways of glucogenesis or ketogenesis.

(*a*) *Glucogenesis.* Most of the amino acids are convertible to carbohydrate ("gluconeogenesis from protein"). The routes vary with the compound concerned, but all converge ultimately at pyruvic acid. The pathways of three glucogenic amino acids to pyruvic acid are particularly direct:

$$\begin{tikzcd}
\text{Glucose} \arrow[d, shift left] \\
\text{Pyruvic acid} \arrow[u, shift left] \arrow[r, shift left] \arrow[d, shift left] & \text{Alanine} \arrow[l, shift left] \\
\text{Oxalacetic acid} \arrow[u, shift left] \arrow[r, shift left] \arrow[d, shift left] & \text{Aspartic acid} \arrow[l, shift left] \\
\alpha\text{-Ketoglutaric acid} \arrow[u, shift left] \arrow[r, shift left] & \text{Glutamic acid} \arrow[l, shift left]
\end{tikzcd}$$

α-Ketoglutaric acid, oxalacetic acid, and pyruvic acid are interconvertible by means of the tricarboxylic acid cycle (p. 8). Pyruvic acid and glucose are connected by the glycolytic series of reactions (p. 6).

It will be noted that the reactions listed above are reversible. This is generally true for the nonessential, glucogenic amino acids, which can be synthesized by the body from ammonia and the α-keto acids provided by carbohydrate. Many amino acids in addition to those shown are glucogenic. Their pathways to pyruvic acid are more devious, however.

It has been estimated that over half of the amino acid constituents of the average animal protein are glucogenic. If a state of total diabetes or phlorizin-diabetes is induced in an experimental animal, it becomes virtually unable to utilize glucose. Preformed carbohydrate or any which may be synthesized from protein is lost practically quantitatively in the urine. If such an animal is fasted, its preformed glycogen is consumed in short order, followed by depletion of its fat stores, at which time the body attempts to support itself by the degradation of tissue protein. All of the protein which is degraded gives rise to urinary nitrogen (urea, NH_3, creatinine, uric acid). Since the animal is in a diabetic state, the glucose derived from the glucogenic amino acids of the degraded protein is excreted, unused, in the urine also. The ratio of glucose to nitrogen in the urine is known as the "G/N ratio" (sometimes D/N, where D = dextrose). This varies with the severity of the diabetes, reaching a maximum value of about 3.65 in the phlorizinized animal. This means that each gram of nitrogen resulting from the degradation of tissue protein has been accompanied by the formation of 3.65 gm. of glucose. Since nitrogen constitutes 16 per cent of the average animal protein (protein = N × 1/0.16), each gram of protein has produced 3.65 × 0.16 = 0.58 grams of glucose. Hence, 58 per cent by weight of the protein is glucogenic. Actually, there are a number of objections to this simple interpretation of the G/N ratio, and it is no longer regarded as an accurate index of the severity of the diabetes or the degree of gluconeogenesis from amino acids or proteins. However, other technics have confirmed the general findings that a large proportion of the amino acids in the average protein is glucogenic.

This phenomenon of gluconeogenesis, which is the only source of carbohydrate in the fasted animal after the pre-existing glycogen reserves have been utilized, takes place in the liver. The 11-oxygenated adrenocortical hormones are apparently required for, and increase the rate of this process (p. 16); in their absence, e.g., in Addison's disease, the blood sugar falls rapidly if exogenous carbohydrate is not supplied (p. 41). Insulin decreases the rate of gluconeogenesis (p. 14); in its absence, e.g., in diabetes mellitus, gluconeogenesis is accelerated, protein catabolism being stimulated, with the production of negative nitrogen balance (p. 77).

(*b*) *Ketogenic Pathway*. The α-keto acids derived from a few amino acids are more closely allied to fats than to carbohydrates. In contrast to the glucogenic amino acids, the ketogenic group produce ketone bodies instead of glucose. In comparison to the glucogenic group, the ketogenic amino acids are in a minority, comprising only phenylalanine, tyrosine, leucine, and isoleucine. Furthermore, certain of these amino acids are metabolized along pathways which are glucogenic as well as ketogenic.

INTERRELATIONS OF THE METABOLISM OF PROTEINS AND OTHER FOODSTUFFS

The major interrelations between proteins, fats, and carbohydrates are outlined in Figure 17. The importance of proteins in the metabolism of all types of compounds, inasmuch as enzymes and many hormones are proteins (or amino acid derivatives) in nature, is too obvious to require elaboration. Synthesis of specialized products, such as purines, pyrimidines, and porphyrins, is likewise an important function of proteins and amino acids, as has been discussed previously. The subject of interest in this section is the relationship between the metabolism of proteins and that of the two other major foodstuffs, as exemplified by direct interconversion and by caloric substitution.

The ketogenic amino acids and certain fragments derived from the metabolism of nonketogenic amino acids form acetate or acetoacetate, thus contributing directly to the synthesis of fatty acids. The glucogenic amino acids can function similarly, but subsequent to initial conversions to pyruvate or keto-acids yielding pyruvate. Certain specialized lipid moieties are also derived from amino acids, viz., serine, ethanolamine, and choline. The pathway from the carbon skeletons of the ketogenic or glucogenic amino acids to the fatty acids is irreversible.

The majority of amino acids can form carbohydrate. In certain cases, viz., alanine, aspartate, and glutamate, the conversion to precursors of carbohydrate is direct; in others the pathway may be quite devious. The conversion is reversible for most of the nonessential, glucogenic amino acids. It must be emphasized in connection with glucogenicity that the α-keto acids produced from the amino acids (pyruvate, oxalacetate, α-ketoglutarate) function not only as raw materials for the synthesis of glucose, but also as catalysts in the channeling of acetyl units from all classes of foodstuffs through the tricarboxylic acid cycle (α-ketoglutarate and pyruvate being easily converted to oxalacetate, the actual catalytic compound of the cycle) for the provision of energy.

The energy content of the diet influences protein metabolism as reflected in the nitrogen balance. Nitrogen excretion is increased when the caloric intake is lowered to sub-maintenance levels. Conversely, increasing the caloric intake causes a decrease in urinary N excretion. At any given adequate level of dietary protein, the nitrogen balance is determined, in part at least, by the caloric intake. This relationship can be demonstrated in another manner. When the caloric intake is adequate, increasing the dietary protein from sub-maintenance levels leads to improvement in N balance. However, if the energy intake is below about 25 per cent of that required, this response to an increased protein intake is not obtained. Under these restricted conditions, the caloric intake is apparently a limiting factor in the utilization of dietary proteins.

On diets grossly inadequate in caloric value, nitrogen equilibrium may still be maintained, especially in obese subjects and if adequate amounts of protein and a small amount of carbohydrate are provided. During the initial days of such a regimen (or of a fast), the N excretion is lower than on subsequent days, owing to utilization of glycogen stores. Later, body fat is utilized for energy, exerting the beneficial effect on N balance indicated above for dietary fat.

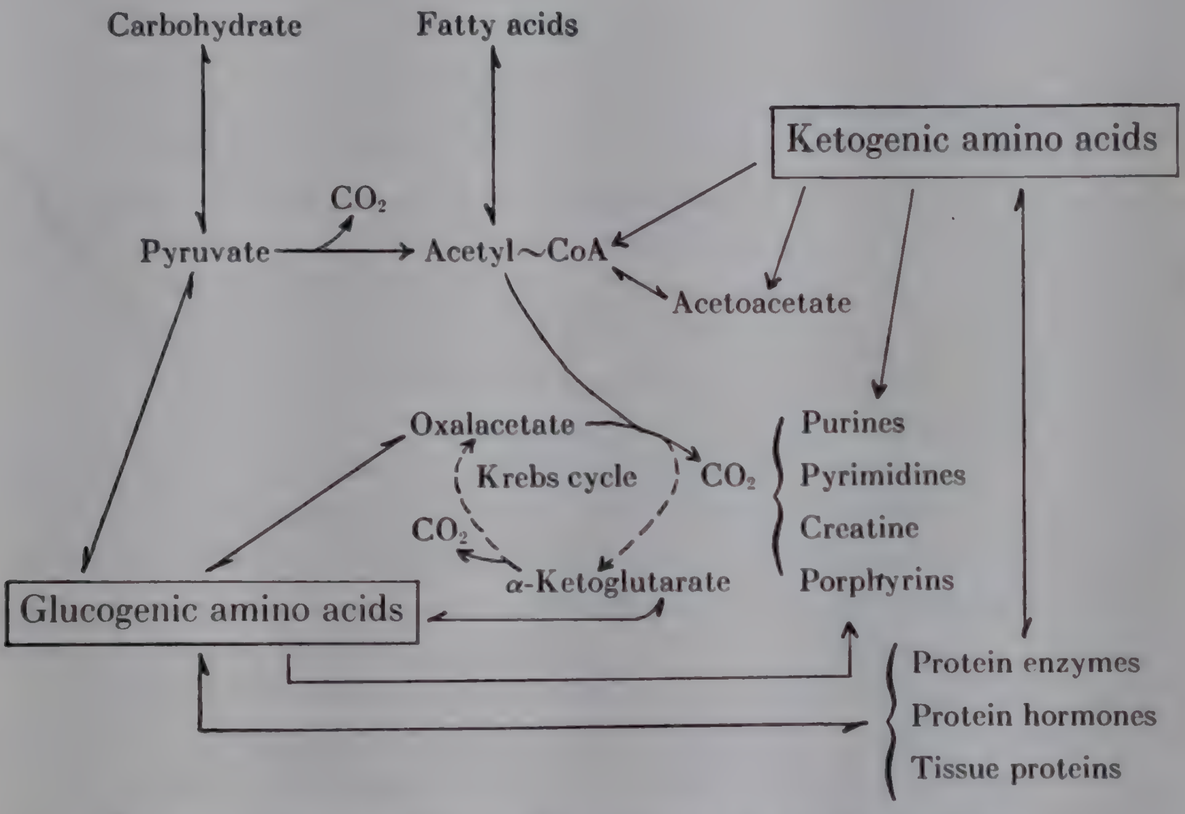
Carbohydrate
Fatty acids
Ketogenic amino acids
CO_2
Pyruvate
Acetyl~CoA
Acetoacetate
Oxalacetate
Krebs cycle
CO_2
CO_2
α-Ketoglutarate
Purines
Pyrimidines
Creatine
Porphyrins
Glucogenic amino acids
Protein enzymes
Protein hormones
Tissue proteins

Fig. 17. Metabolic interrelations of proteins with other foodstuffs.

These effects of the energy content of the diet are apparently largely independent of the nature of the energy-providing materials, being essentially identical, except temporarily (see below), with equicaloric amounts of carbohydrate, fat, or alcohol. They are not influenced by the time of feeding of the latter in relation to that of the protein. The mechanism of production of these effects is not known.

Dietary carbohydrate exerts an influence on protein metabolism not shared by other substances. This is independent of its caloric effect, which is shared equally by fat and alcohol; it is referred to as the specific "protein-sparing" action of carbohydrate. It manifests itself in several ways:

1. Isocaloric substitution of fat for carbohydrate in the diet is followed by an increase in nitrogen excretion. This is of relatively brief duration, however, the original level of N balance being restored after several days. This effect is observed whether or not the energy and protein contents of the diet are adequate.

2. If the protein and carbohydrate components of an adequate diet are ingested separately, at wide time intervals, there is a transitory increase in N excretion. No such effect is exhibited by fat.

3. On an exclusively fat diet, the nitrogen output is the same as during starvation, whereas administration of carbohydrate reduces the nitrogen output.

The mechanism underlying this phenomenon is not known for certain. It is apparently not mediated by hormonal influences (pituitary, thyroid, insulin) nor by improved synthesis of amino acids or of protein. It is probably a reflection of the fact that carbohydrate, in addition to its function as a fuel, also provides the major source of oxalacetate for the tricarboxylic acid cycle and the carbon skeletons of nonessential amino acids. In the absence of carbohydrate, protein is called upon specifically to assume these functions, since fat cannot. The increased degradation of protein and loss of nitrogen are necessarily greater than would occur if fat were omitted from the diet instead of carbohydrate.

ENDOCRINE INFLUENCES IN PROTEIN METABOLISM

Anabolism (synthesis) and catabolism (degradation) of proteins are influenced, if not indeed regulated by certain hormones, which also influence the interrelationships between the metabolism of protein and of carbohydrate. The most important of these are the growth hormone (anterior pituitary), the adrenal 11-oxysteroids, thyroxine, androgens, and insulin.

Growth Hormone. This exerts a protein anabolic effect. Its administration is followed by diminished urinary excretion of nitrogen (positive N balance), with a decrease in plasma urea and amino acids and increased deposition of protein in the tissues. Insulin is necessary for the production of this effect. Somatic growth is induced in prepubertal children.

Androgen. Testosterone, too, exerts a protein anabolic effect, as do androstenediol and androstenedione, but androsterone and dehydroisoandrosterone exhibit little such action. Administration of testosterone is followed by decreased urinary excretion of nitrogen, positive N balance, stimulation of somatic growth in prepubertal children (adolescent growth spurt), and increased weight (i.e., protein deposition) not only of specific target tissues (e.g., prostate, seminal vesicles), but also of other organs, particularly the kidneys (renotropic action). Castration is followed by increased urinary excretion of creatine; this is reversed by testosterone. Methyltestosterone, a synthetic androgen which also exerts a protein anabolic effect, increases urinary excretion of creatine. It is believed to stimulate synthesis as well as storage of creatine, whereas testosterone stimulates only its storage.

Adrenal 11-Oxysteroids. Under usual experimental and clinical conditions, the 11-oxygenated adrenocortical hormones apparently exert a protein catabolic effect, i.e., they cause increased urinary excretion of nitrogen and a negative N balance. This effect is intimately related to their action in increasing gluconeogenesis (p. 16), which probably involves increased deamination of amino acids and increased conversion of the nonnitrogenous residues to glucose (or glycogen). This stimulation of gluconeogenesis is antagonized by insulin (p. 14). It is interesting that exogenous protein is apparently not affected in this manner. Studies with labeled amino acids suggest that these hormones decrease synthesis of protein, indicating that their action in this connection may be "antianabolic" rather than, or in addition to, catabolic. They also cause increased urinary excretion of uric acid, apparently a reflection of increased catabolism of purines (i.e., nucleic acids). These hormones depress growth of embryonic tissue (chick) and tumors. They inhibit healing of wounds, interfering with the formation of granulation tissue and ground substance.

There is evidence, however, that when administered in relatively small amounts, presumably approximating physiological quantities, these hormones may exert a protein anabolic effect, with reduced urinary nitrogen excretion and positive N balance.

Insulin. Insulin is necessary for the protein anabolic effect of growth hormone, and antagonizes the gluconeogenic action of adrenocortical hormones. There is some indication that insulin may stimulate growth (protein anabolism) in the absence of growth hormone.

Thyroxine. In physiological doses, thyroxine exerts a protein anabolic effect, as indicated by a positive N balance, especially in the growing organism. This action would be inferred from the fact that removal of the thyroid in early life results in retardation of growth, which can be corrected by administration of thyroid hormone. Thyroidectomy (and hypothyroidism) is followed by accumulation in the extracellular fluids of a mucoprotein, rich in hyaluronic acid and similar to fetal mucin in composition (myxedema).

In unphysiological (large) doses, thyroxine exerts a protein catabolic effect, with increased urinary excretion of creatine (creatinuria), resulting from excessive catabolism of muscle tissue and also, perhaps, from a decreased capacity for prompt resynthesis of phosphocreatine during the recovery stage of muscle contraction.

NITROGENOUS CONSTITUENTS OF THE BLOOD

The blood plasma normally contains a number of nitrogenous substances. These may be classified as (1) proteins and (2) nonprotein nitrogenous substances.

The Plasma Proteins

Segregation of discussion of the plasma proteins is justified by two considerations. First, due to their intimate relation to protein metabolism in the liver, as well as to their interaction with other tissues throughout the body, the plasma proteins occupy a central position in the metabolism of protein. The second consideration is entirely practical: the plasma proteins happen to be the most conveniently obtainable sample of protein available in the body. Because of the interrelations between the proteins of plasma and the tissues, a great deal can be learned concerning the general status of protein metabolism from examination of the plasma proteins.

Identity and Properties. The heterogeneity of the plasma proteins is readily demonstrated in the ultracentrifuge. In addition to fibrinogen, albumin, and a group of globulins sedimenting at a rate between these two, the ultracentrifugal analysis of plasma reveals a group of very rapidly sedimenting globulins and lipoproteins, the sedimentation of which varies markedly with the density of the medium. This heterogeneity is reflected in the wide range of molecular weights, i.e., sizes, of the various plasma proteins. Albumin, the smallest, has a molecular weight of about 69,000, α-globulins 200,000 to 300,000, β-globulins 150,000 to 1,300,000, γ-globulins 150,000 to 300,000, and fibrinogen, 400,000. Inasmuch as osmotic pressure is a function of the number of active particles (e.g., molecules) in solution, gram for gram, albumin has considerably more influence on osmotic pressure than do the other plasma proteins. Moreover, being the smallest of these molecules, it is the first to escape from the blood stream when the permeability of the capillary walls is increased, e.g., in inflammation.

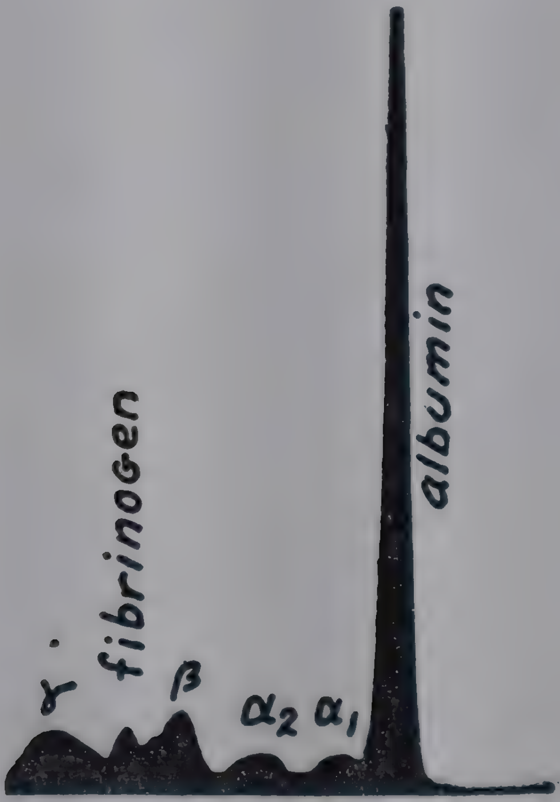

Fig. 18. Electrophoretic patterns of normal plasma.

The degree of resolution of the plasma proteins in the ultracentrifuge is greatly inferior to that obtainable with electrophoresis. An electrophoretic diagram of normal plasma is shown in Figure 18. It will be noted that, in addition to albumin and fibrinogen, this technic resolves the globulins into four groups (α_1, α_2, β, and γ).

Most of the clinical data on the plasma proteins have been obtained by the method of salting-out. Precipitates or supernatant solutions produced by the addition to the plasma of certain concentrations of salts are analyzed for protein content. Before the advent of the electrophoretic technic, clinical analyses of plasma by salting-out were performed according to the method of Howe. The Howe fractions have since been shown to disagree markedly with the results of electrophoresis. A comparison is shown in Table 3 of the Howe method and a modern adaptation (Milne) which yields fractions more closely related to those obtained electrophoretically. It would appear that, with the Howe procedure, which is widely used, the "albumin" fraction contains about 1 gram of globulin. With the "Milne" procedure, pseudoglobulin corresponds rather closely to the α-globulins, and euglobulin to the β- and γ-globulins.

The fractions of the plasma proteins obtained by ultracentrifugation, electrophoresis, or salting-out are chemically heterogeneous. For scientific and other purposes, methods have been elaborated which permit fractionation of the plasma proteins to the point of isolation of relatively homogeneous chemical constituents. These methods are of interest not only in that they permit, for the first time, a closer study of the physicochemical and physiological properties of the individual proteins of the plasma, but also because they have made possible the large-scale production of plasma protein products for therapeutic use, viz., fibrin film and foam (surgery), purified albumin (shock), and globulin (measles, hepatitis, poliomyelitis).

Table 3. Plasma Protein Concentrations

	SALTING OUT			
PROTEIN	HOWE	MILNE (modified)	ELECTROPHORESIS	
	(grams %)	(grams %)	(grams %)	(% of total)
Total	6–8 (7)	6–8 (7)	6–8 (7)	100
Albumin	4.7–5.7 (5.2)	3.6–4.5 (4.0)	3.0–4.1 (3.6)	51
Globulin	1.3–2.5 (2.0)	2.1–4.2 (3.2)	2.7–3.8 (3.2)	45
Pseudo-	(1.8)	0.8–1.9 (1.3)	α (1.0)	14
Eu-	(0.2)	1.3–2.5 (1.9)	β (1.3)	19
			γ (0.9)	12
Fibrinogen	0.2–0.4 (0.3)	0.2–0.4 (0.3)	(0.3)	4

These procedures are based upon fractional precipitation of the plasma proteins with ethanol at low temperatures and low salt concentration. Proper adjustment of the concentrations of protein, salts, and ethanol, temperature, and pH, permits the isolation of six fractions ("Harvard" or "Cohn" fractions), each of which can be subfractionated by appropriate procedures into relatively pure components. The relative amounts of the plasma fractions so obtained, their natural functions, and their clinical uses, are indicated in Figure 19 and Table 4.

Two lipoproteins, an α_1- and a β_1-globulin, have been isolated by the methods described above. However, there is evidence that a larger number of such proteins may be present in plasma. By the addition of salt to plasma, its density may be elevated to the point where the lipoproteins (having a lower density than ordinary proteins due to their lipid content) will migrate toward the axis instead of toward the periphery in the ultracentrifuge. Whereas the rates of sedimentation of particles in conventional ultracentrifugation are expressed in Svedberg units ($S = 1 \times 10^{-13}$ sec.), those of the lipoproteins observed in the flotation method are expressed in "negative" S units, or "flotation" S units, S_f. In these terms, particles have been found distributed over the range $S_f = 2–40{,}000$, although it is suspected that substances migrating at $S_f > 17$ may represent aggregates of fat and lipoprotein (p. 85). The purported relationship between the concentrations of the S_f 12–20 lipoproteins and the incidence of atherosclerosis is the subject of active investigation (p. 111).

Table 4. Identities and Properties of Major Plasma Proteins

(Data obtained from various publications of the University Laboratory of Physical Chemistry Related to Medicine and Public Health, Harvard University).

PROTEIN OR GROUP	GRAMS/ LITER OF PLASMA	MOL. WT.	SPECIAL PROPERTIES
Fibrinogen	2	400,000	
γ-Globulin	5	156,000	Antibodies
γ-Globulin	1	300,000	Antibodies
β_1-Globulin	2	1,300,000	β_1-Lipoprotein
β_1-Globulin	1	500,000–1,000,000	Lipid-poor euglobulins
β_1-Globulin	2	150,000	
β_2-Globulin	2	150,000	Isoagglutinins
Prothrombin			
α_1-Bilirubin globulin			Transports bilirubin
α_1-Globulin	2	200,000	α_1-Lipoprotein
α_2-Globulin	1	300,000	Glycoproteins, mucoproteins, esterase
β_1-Globulin	2	90,000	Metal-combining globulin (siderophilin).
Albumin	32	69,000	

Metabolism. From isotopic and other evidence, it has been concluded that the liver is the chief, if not the sole site of formation of albumin, fibrinogen, prothrombin and α- and β-globulins. It is currently believed that the γ-globulins are synthesized mainly in plasma cells. They may be stored for a time in the reticuloendothelial (also lymphoid?) cells, which also may act to split aggregated antigens into immunochemically active forms. The possible role of lymphocytes in the elaboration of γ-globulins is not clear at this time.

The nutritional status of the individual with respect to protein has a profound effect on the synthesis of plasma proteins, both directly, in terms of provision of raw materials for synthesis, and indirectly, due to the deleterious effect of protein deprivation on the liver. It is of interest that protein deprivation has its most marked effect on the levels of plasma albumin and γ-globulins, a decrease in the former of sufficient severity leading to edema (p. 155), a decrease in the latter fraction resulting in impaired resistance to infections.

It has been estimated, from studies with isotopes, that a 70 kg. man synthesizes and degrades approximately 15 to 20 gm. of plasma protein per day. Not all of the fractions turn over at the same rate, however. Albumin and γ-globulins are synthesized at somewhat slower rates than the α- and β-globulins. Under optimum conditions, i.e., adequate protein supply and marked stimulus to synthesis, much more can undoubtedly be formed. It has been found that normal dogs, depleted of plasma proteins (plasmapheresis), are capable, under these conditions, of regenerating about 90 per cent of their total plasma proteins weekly. For a 70 kg. man, this would amount to 25 to 30 gm. daily.

The plasma proteins, among their other functions (discussed below), serve as a source of protein nutrition for the tissues. Indeed, a dynamic equilibrium exists between the proteins of the tissues and those of the plasma, each group sustaining the other when the need arises. In conditions of protein deprivation, however, the normal level of the plasma proteins is apparently guarded more zealously than that of the tissue proteins, since the latter are degraded to provide for the former. As a result, considerable loss of tissue protein may occur with minimal decrease in the concentrations of plasma proteins. It has been calculated that a decrease in total circulating plasma protein of 1 gm., in hypoproteinemia due solely to protein deprivation, represents a concomitant loss of 30 gm. of tissue protein.

Observations in experimental animals indicate that the plasma proteins circulate within a much larger volume than that of the blood plasma, passing continually from the plasma and other extracellular fluids into the lymph and back again. In the dog, about 50 per cent of the total traverses the thoracic duct daily. It appears, also, that the intravascular plasma protein is in dynamic equilibrium with the extravascular plasma protein, a decrease in one compartment resulting in movement in that direction from other compartments.

Functions. (1) NUTRITIVE. The nutritive functions of the plasma proteins are probably attributable largely to the albumin fraction, due to its quantitative dominance. Intravenously administered albumin has been shown in fact to be efficiently, although slowly, utilized in humans.

(2) WATER DISTRIBUTION. Because of the relative impermeability of the capillary walls to colloids, the colloid osmotic pressure of the plasma proteins, which normally ranges from 24 to 30 mm. Hg, plays an important part in the maintenance of the normal distribution of water between the blood and the tissues. The osmotic pressure of blood plasma is approximately 7 atmospheres, being dependent upon its crystalloid and colloid content. Because of the fairly high degree of diffusibility of the crystalloid constituents, the concentrations of these substances in plasma and tissue fluids are practically identical; minor differences exist because of the effect of a Donnan equilibrium. It may therefore be considered that, in the resting state, the crystalloid osmotic pressure of the plasma is balanced by that of the tissue fluids.

There is considerable disparity, however, between the protein content of plasma and that of tissue fluid, the latter varying in different situations due to varying degrees of capillary permeability, as demonstrated by Starling. Thus the lymph flowing from the liver contains 6 to 7 gm. per cent of protein, that from the extremities only 2 to 3 gm. per cent. Because of this difference, the colloid osmotic pressure of the plasma (24–30 mg. Hg) exerts a force which tends to direct the flow of water from the tissues into the blood. This force, the colloid osmotic pressure or oncotic pressure of the plasma, is a function largely of the molecular size of the various proteins. It is influenced consequently much more by albumin than by globulin, 2.4 to 3.9 times as much, according to different observers, because of the smaller size of the albumin molecule (molecular weight of serum albumin about 69,000; serum globulins, 150,000–1,300,000).

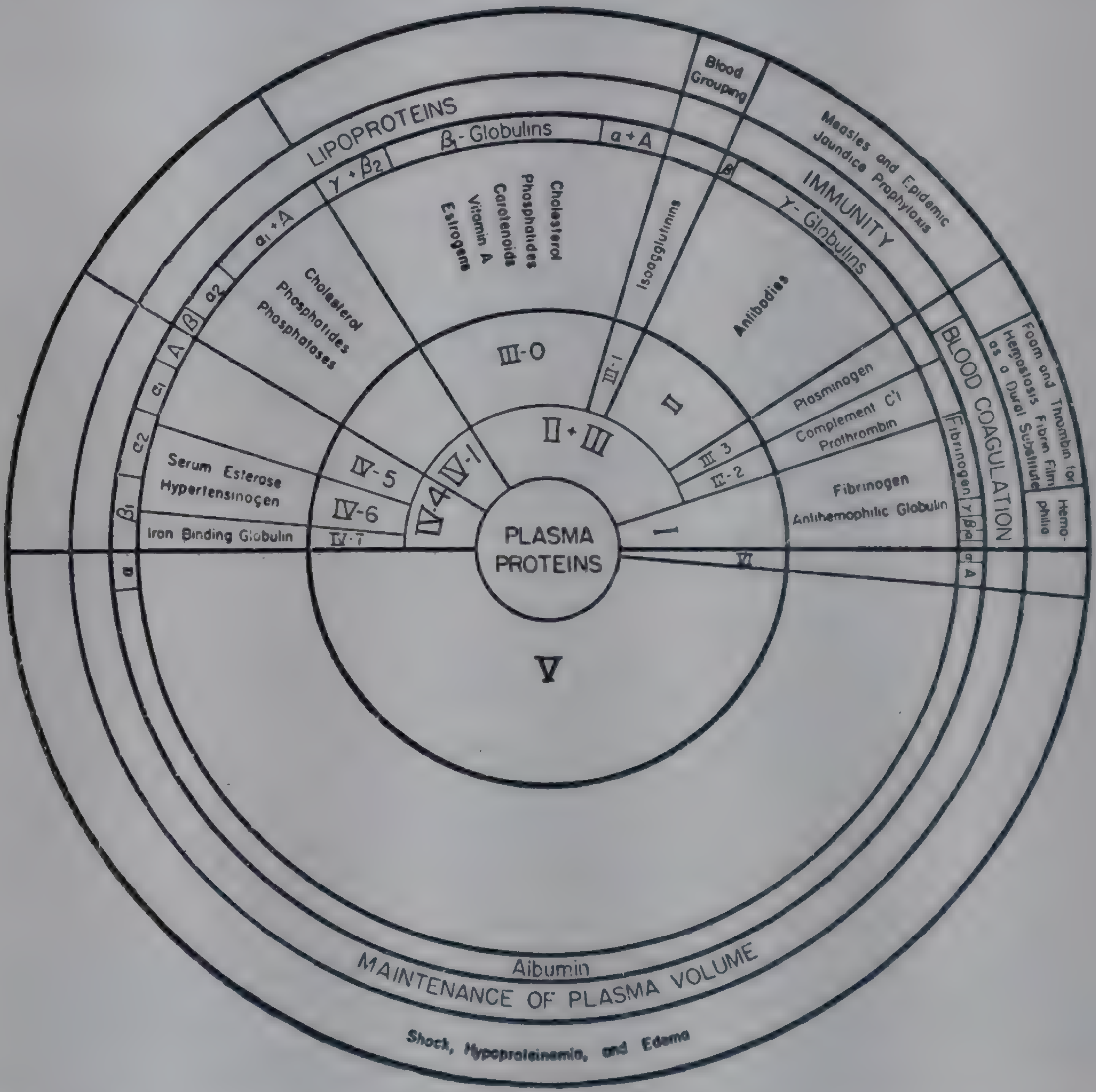
LIPOPROTEINS
β_1- Globulins
a + A
$\gamma + \beta_2$
$a_1 + A$
a_2
β
A
a_1
a_2
β_1
a
Cholesterol
Phosphatides
Carotenoids
Vitamin A
Estrogens
Cholesterol
Phosphatides
Phosphatases
Serum Esterase
Hypertensinogen
Iron Binding Globulin
III-0
IV-5
IV-6
IV-7
IV-4
IV-1
II + III
PLASMA
PROTEINS
Blood
Grouping
Isoagglutinins
III-1
β
Measles and Epidemic
Jaundice Prophylaxis
IMMUNITY
γ- Globulins
Antibodies
II
Plasminogen
Complement C'1
Prothrombin
III 3
III-2
Fibrinogen
Antihemophilic Globulin
I
VI
BLOOD COAGULATION
Fibrinogen
γ
β
a
a
A
Foam and Thrombin for
Hemostasis Fibrin Film
as a Dural Substitute
Hemo-
philia
V
Albumin
MAINTENANCE OF PLASMA VOLUME
Shock, Hypoproteinemia, and Edema

Fig. 19. Natural functions, clinical uses, and fractionation of the plasma proteins (from Edsall: Advances in Protein Chemistry, Vol. 3, Academic Press, 1947).

Thus, albumin, comprising about 60 per cent of the total proteins, is responsible for about 80 per cent of the oncotic pressure of human plasma. By direct measurement, the colloid osmotic pressure of normal plasma or serum has been found to vary from 280 to 470 mm. H_2O. Under normal conditions, this force, which tends to direct the flow of water from the tissues into the blood, is counterbalanced by the opposing force of the capillary blood pressure (13–35 mm. Hg). Because of the drop in blood pressure from the arteriolar to the venous end of the capillary, fluid tends to pass from the blood to the tissues on the arteriolar side of the capillaries and from the tissue spaces to the blood at the venous end.

Passage of fluid in the latter direction is also favored by the increased colloid osmotic pressure of the blood plasma at the venous end of the capillary, which results from the increased concentration of plasma proteins incident to the loss of fluid in the proximal end of the capillaries. Under conditions of active tissue metabolism the osmotic pressure of the tissue fluids is raised by the decomposition of carbohydrates, proteins and fats into relatively much smaller molecules, thus temporarily aiding in the abstraction of water from the blood plasma.

(3) ACID-BASE BALANCE. From a quantitative standpoint, the buffering capacity of the plasma proteins is not very great. In whole blood, however, the combined buffering action of the hemoglobin and plasma proteins is as important as that due to the bicarbonate and other inorganic buffer systems of the blood (p. 325).

(4) TRANSPORT. The lipid-transporting functions of the plasma proteins are discussed in detail elsewhere (p. 101). In addition to the lipids, the lipoproteins transport certain lipid-soluble compounds, such as the steroid hormones (e.g., estriol) and the fat-soluble vitamins (e.g., A, D, and E). Bilirubin is associated with a fraction of the α-globulins (and is also bound by albumin). β_1-Metal combining globulin (siderophilin) is responsible for the transport of iron in the plasma. This protein can also combine with copper; however, a specific protein (caeruloplasmin) also appears to be elaborated for this purpose. Approximately half of the calcium of plasma is bound to protein in a linkage which is relatively weak in comparison to the metalloproteins just mentioned. Thyroxine also appears to be transported in loose association with the plasma proteins. In addition to the more or less specific transport functions already mentioned, due in most cases to the various globulins, plasma albumin has been found to exhibit considerable affinity for anions and cations having a wide variety of structures. It is probable that many drugs and dyes are transported in the plasma in combination with albumin.

(5) COAGULATION. In addition to fibrinogen and prothrombin (Fig. 19), plasma contains a number of other components which participate in the process of blood coagulation, viz., Ac-globulin, serum prothrombin conversion accelerator, antihemophilic globulin, antithrombin, heparin cofactor, antithromboplastic lipoprotein, plasminogen, and plasmin inhibitor.

(6) IMMUNITY. Reference has been made to the immunological functions of the plasma proteins. The γ-globulins contain a large number of antibodies, among which may be mentioned those against influenza, mumps, poliomyelitis, measles, infectious hepatitis, typhoid, whooping cough, and diphtheria. The β_2-globulin fraction contains the antibodies to the isoagglutinins, the antigens responsible for the various blood groups in man. Two of the components of complement, a cofactor necessary in certain types of immunological reactions, are proteins of the globulin type.

(7) ENZYMES. The protein constituents of the plasma include a number of enzymes, the physiological functions of which are obscure in most cases, but some of which are of clinical diagnostic importance, e.g., amylase (p. 490), lipase (p. 491), phosphatases (pp. 249, 252).

Nonprotein Nitrogen

The nonprotein nitrogen (NPN) of the blood, that portion of the nitrogenous substances not precipitated by the usual protein precipitants, includes urea, uric acid, amino acids, creatine, creatinine, ammonia, and a fraction designated "undetermined nitrogen," which consists perhaps of polypeptides and other aggregations of amino acids. From a metabolic standpoint, the nonprotein nitrogenous constituents of the blood are usually of greater clinical interest than the plasma proteins, since they represent products of the intermediary metabolism of ingested and tissue protein. The normal NPN of whole blood is usually 25 to 35 mg. per 100 cc., figures as high as 40 mg. occurring occasionally, and as low as 16 mg. at times in normal pregnancy.

Urea. The blood urea normally ranges from 20 to 35 mg. per 100 cc., the urea N being 9 to 17 mg. (46.6% of the total urea molecule). The extreme normal limits under conditions of very low to very high protein intake are 5 to 23 mg. urea N per 100 cc. Values of 5 to 12 mg. are common in the last months of normal pregnancy. Urea is the chief end-product of protein metabolism and is formed normally largely if not entirely in the liver, by a mechanism described elsewhere (p. 136).

The blood urea nitrogen may rise following a meal high in protein and tends to maintain a relatively higher level in individuals on a high protein diet than in those on a low protein intake. It is essential, therefore, in making comparisons between blood urea values, that a standard diet be administered and that the specimen be taken in the fasting state.

Urea is an extremely diffusible substance and, as such, exists in all body fluids in practically the same concentration. Thus it is present in the spinal fluid, saliva, exudates, and transudates in approximately the same amount as in blood. It is eliminated chiefly in the urine, but considerable amounts may be lost through the skin if perspiration is active.

Uric Acid. The uric acid content of normal blood is 2 to 4 mg. per 100 cc. and of plasma or serum, collected anaerobically, 2 to 6 mg. per 100 cc. The latter values are more significant clinically than the former. Uric acid is, in man, the chief end-product of purine metabolism, and will be discussed in connection with the metabolism of nucleic acids (p. 199).

Creatinine. The creatinine content of normal blood ranges from 1 to 2 mg. per 100 cc. It is the anhydride of creatine, which is present in the muscles. Creatinine is readily diffusible and is excreted largely by the kidneys.

The methods usually employed for the determination of blood creatinine are nonspecific in nature, although specific (enzymatic) methods are available. However, whatever may be the exact nature of the substances in the blood which respond to the reaction for creatinine, so remarkable is their constancy in health and so specific are variations in their concentration in disease that the question as to whether or not this nitrogen fraction is in reality creatinine is of more academic than practical clinical interest.

Amino Acids. The amino acid nitrogen content of normal blood is 5 to 8 mg. per 100 cc., the average concentration in plasma being about 3.5 mg. per 100 cc. It is derived from that portion of the amino acids absorbed from the intestine which has escaped deamination and synthesis into protein in the liver, and from amino acids resulting from breakdown of tissue protein which have not as yet undergone deamination or synthesis. A rise in the amino acid concentration of the blood occurs following the ingestion of proteins. A decrease follows administration of insulin, growth hormone, or testosterone in normal subjects, due presumably to stimulation of protein synthesis. A similar effect is apparently produced by epinephrine, the mechanism being unknown, and by administration of glucose (insulin increase?).

Ammonia. The ammonia nitrogen content of normal blood is 0.3 to 0.97 mg. per 100 cc. It is produced as a result of the deamination of amino acids by the liver and represents largely that portion which has not been converted into urea or utilized for other purposes (p. 135).

Undetermined Nitrogen (Rest Nitrogen). After all of the known nonprotein nitrogenous constituents of the blood have been determined, including those mentioned above and others, such as creatine (2–7 mg. per 100 cc.), of no apparent clinical significance, there remains a considerable amount of nonprotein nitrogen of undetermined composition termed "rest nitrogen" or "undetermined nitrogen." This may constitute as much as 45 per cent of the total nonprotein nitrogen, ranging normally from 5 to 18 mg. per 100 cc. and residing chiefly in the corpuscles. Since its nature is unknown, its metabolic significance and its derivation are conjecturable. However, it may bear an important but as yet unknown relationship to the toxic manifestations of certain disease states, such as eclampsia and uremia.

NITROGEN EXCRETION

Under normal conditions the urine contains an extremely small amount of albumin which usually cannot be detected by ordinary qualitative methods. The excretion of albumin by essentially normal individuals (functional, orthostatic or adolescent albuminuria) will be considered in the discussion of albuminuria (p. 172). From a practical viewpoint, with certain reservations, therefore, it may be stated that protein is not normally present in the urine.

Practically all of the nonprotein nitrogenous constituents of the blood are eliminated by the kidneys.

Urea. The quantity of urea excreted in the urine depends largely upon the protein intake. The percentage of the total urinary nitrogen represented by urea likewise varies directly with the amount of protein ingested. The lower the protein intake the greater is the relative proportion of products of endogenous metabolism such as creatinine and uric acid, although the actual amount of the former is unaltered and the latter decreased. The relative proportion of ammonia nitrogen is likewise greatly increased under such circumstances. Upon a high protein intake (120 gm.), urea may constitute approximately 90 per cent of the total urine nitrogen; upon an intake of 6 gm. of protein this figure is reduced to about 60 per cent (Folin). With an average diet, approximately 30 gm. of urea are eliminated in twenty-four hours, constituting about 50 per cent of the total urinary solids (see Table 5, p. 187).

It is evident that the total urinary nitrogen, as well as the urea nitrogen, depends upon the nitrogen intake and, to be of any clinical significance, must be regarded in its relation to the latter. Furthermore, since nitrogen balance may be established with varying levels of nonprotein nitrogen in the blood, the isolated determination of total urinary nitrogen or urea nitrogen is of little clinical value under ordinary circumstances.

The mechanism of renal excretion of urea and the clinical significance of abnormalities in this function are considered in detail in the discussion of renal function (p. 599).

Uric Acid. The quantity of uric acid present in the urine of normal individuals depends upon the amount of nucleoprotein ingested (exogenous uric acid) and upon the amount formed as a result of the metabolism of tissue nucleoprotein (endogenous uric acid). This subject is discussed elsewhere (nucleic acid metabolism, p. 199).

Creatine and Creatinine. Creatine, derived from glycine, arginine (amidine group), and methionine (methyl group), is not a waste product, but is of fundamental importance in the phenomenon of muscle contraction (as phosphocreatine). The capacity of the muscles for contraction is related to their phosphocreatine content, the force of contraction is proportional to the extent of breakdown of the latter, and restoration of excitability in the muscle after contraction is dependent upon resynthesis of the hydrolyzed phosphocreatine (p. 11).

Creatine is present in the urine of prepuberal children (4.2 mg. per kilogram daily), during pregnancy and the puerperium (two to three weeks), and occasionally in normal, nonpregnant women, but is absent from the urine of normal males on a balanced diet. It may appear in the urine of normal subjects maintained on a very low carbohydrate intake and during fasting periods (up to 100 mg. creatine daily), and is believed to be derived from excessive catabolism of muscle tissue. Creatine is present in the blood almost entirely in the erythrocytes (2–7 mg. %); when its concentration in the plasma exceeds 0.6 mg. per 100 cc. it almost invariably appears in the urine. This suggests that it is completely reabsorbed in the renal tubules at lower concentrations.

When 1.32 or 2.64 gm. of creatine hydrate (equivalent to 1 and 2 gm. of creatine) are ingested by normal adults, about 80 per cent is retained by males and about 70 per cent by females, about 20 per cent and 30 per cent respectively being excreted in the urine during the next twenty-four hours. This forms the basis of the creatine tolerance test, which is of value clinically in demonstrating excessive excretion of creatine in the absence of spontaneous creatinuria under ordinary dietary conditions. It is also of value in affording a quantitative index of the functional state of the muscles in various myopathies and other conditions (p. 181).

Creatinine (creatine anhydride) is a waste product derived from creatine, and is therefore usually of endogenous origin. Normal adult males excrete 1.5 to 2 gm. and females 0.8 to 1.5 gm. daily, the amount eliminated being uninfluenced by the urine volume or the character of the diet if the latter is adequate and contains no creatinine or creatine. Folin found that upon a diet containing 118 gm. of protein and 2786 calories, 0.58 gm. of creatinine was eliminated, constituting 3.6 per cent of the total urinary nitrogen; upon a diet containing 6 gm. of protein and 2153 calories, 0.60 gm. of creatinine was eliminated, constituting 17.2 per cent of the total urinary nitrogen (See Table 5, p. 187). Because of this constancy of elimination, the determination of the total creatinine excretion has been employed as a means of checking the accuracy of twenty-four-hour urine collections. If marked variations in urinary creatinine are observed that do not depend upon corresponding changes in weight or surface area, one may assume that there has been some error in the collection of the urine specimens. Because of its origin from creatine, the quantity of creatinine excreted in the urine is an index of the level at which the body maintains its phosphocreatine and not of the rate of endogenous protein metabolism. The amount of creatinine excreted in the urine in twenty-four hours corresponds to about 2 per cent of the creatine in the body.

The term "creatinine coefficient" is used to indicate the number of milligrams of creatinine (plus creatine) nitrogen excreted per kilogram of body weight in twenty-four hours. Normal values range from 7.5 to 10 (20–26 mg. creatinine) for men and 5 to 8 (14–22 mg. creatinine) for women. The creatinine coefficient may be assumed to be an index of the amount of active muscle tissue in the body. It is believed by some that muscular exercise has no influence in this connection, but others believe that although the daily elimination is constant, the urinary creatinine is increased during the period of muscular activity, being derived from creatine liberated during this period.

Amino Acids. Amino acids are eliminated in both the combined and the free state. In normal adults the total urinary amino acid nitrogen varies from 0.5 to 1.0 gm. daily, constituting from 2 to 6 per cent of the total urinary nitrogen. The free amino acid nitrogen ranges from 0.1 to 0.15 gm. daily, or 0.5 to 1.0 per cent of the total urinary nitrogen. Under normal conditions, amino acids pass freely from the blood plasma into the glomerular filtrate, but are very efficiently reabsorbed by the renal tubular epithelium and returned to the blood stream. Even when the plasma level is elevated by intravenous injection of amino acids, comparatively little escapes in the urine, usually not over 5 per cent of the amount administered.

Histidine, an amino acid not ordinarily excreted in significant amounts, appears in the urine at about the fifth week of pregnancy and disappears from the urine within a few days after delivery. There is evidence that the activity of liver histidinase is inhibited during this period, failure of the enzyme to catalyze the catabolism of histidine accounting for the presence of this amino acid in the urine. The amount excreted is increased by a high intake of protein.

Ammonia. The daily output of ammonia in the urine varies from 0.5 to 1.0 gm., comprising 2 to 5 per cent of the total urinary nitrogen. As stated elsewhere (p. 136), urinary ammonia is formed in the kidneys, being derived from amino acids and glutamine through the activity of the epithelial cells of the renal tubules (p. 623). This subject is discussed in detail elsewhere (acid-base balance, p. 331).

Urinary ammonia varies in accordance with the body's needs for excretion of acid with, at the same time, the maximum conservation of fixed base. Exceptions to this rule include acidosis accompanying renal disease, in which the ammonia-forming function of the renal epithelium is impaired (p. 623). Urinary ammonia is increased in normal subjects following ingestion of acid-forming foods, mineral acids or their ammonium, calcium or magnesium salts, by a low carbohydrate intake (ketosis), and in normal pregnancy. It is decreased following ingestion of alkalis or base-forming foods and during the "alkaline tide" accompanying gastric secretion of free HCl (p. 571).

ABNORMAL PROTEIN METABOLISM

Investigation of protein metabolism clinically is restricted almost entirely to the study of the protein and nonprotein nitrogenous constituents of the blood and urine.

Total Plasma (Serum) Protein. Certain generalizations may be made concerning the trends of abnormalities in disease. These are most often in the direction of a decrease in total protein concentration, due invariably to decrease in albumin. Although the globulin fraction may occasionally also be low, it generally exhibits a simultaneous increase. The causes and significance of these changes in individual protein components will be discussed subsequently.

The degree of hypoalbuminemia usually exceeds that of the frequently coexisting hyperglobulinemia, the total protein concentration being, therefore, subnormal. However, in certain cases, particularly of multiple myeloma, viral and protozoal diseases, and, occasionally, hepatic cirrhosis, the converse may occur, with consequent increase in total protein concentration, e.g., as high as 16 gm./100 cc. in multiple myeloma. Regardless of their magnitude, the changes in these two fractions in opposite directions tend to offset each other and, obviously therefore, the total protein concentration may not reflect either the nature or the extent of an existing abnormality, and may, indeed, even fail to indicate its presence. Some type of fractionation procedure (p. 143) is required for these purposes. This may be illustrated by the observation that the total plasma protein concentration was found to be within normal limits in about 90 per cent of cases of ordinary malnutrition, the albumin being slightly decreased and the globulin correspondingly increased.

There are other pitfalls in the interpretation of plasma protein values. On standing erect for one-half hour, the concentration may increase about 1 gm., i.e., from 6.7 gm. to 7.5 gm./100 cc., due to hemoconcentration incident to increased transudation from capillaries in dependent portions (increased venous pressure). It must be borne in mind, too, that in many disease states (e.g., diabetes mellitus, glomerulonephritis, protracted diarrhea, or vomiting), hemoconcentration due to dehydration may mask the presence or extent of an existing serious plasma protein deficit. The true situation is revealed only by determining the total circulating plasma protein, i.e., plasma volume × protein concentration. For example, during a period of protein depletion in dogs, the plasma protein concentration fell only from 7 gm. to 5.9 gm./100 cc. (about 15 per cent) although the total amount of circulating plasma protein fell from 16.5 gm. to 6.5 gm. (about 60 per cent). Similar observations have been made in inmates of prison camps. However, measurement of plasma volume is not always feasible, and is certainly not necessary in every case.

Clinical investigation of plasma proteins consists usually in chemical determination of total protein and of albumin (salt fractionation), the total globulin being obtained by difference. With few exceptions (e.g., fibrinopenia of pregnancy; congenital fibrinogenopenia), quantitative determinations of fibrinogen are of little or no diagnostic or other clinical significance. In certain cases, partitioning of the globulins may be desirable, i.e., into pseudoglobulin (corresponding to α-globulins) and euglobulin (β- plus γ-globulins) fractions (p. 143). The γ-globulins may be determined, roughly, by means of zinc sulfate turbidity measurements (p. 163). Increases in γ- and β-globulins are also reflected, roughly, in abnormalities in thymol turbidity (p. 164) and cephalin-cholesterol flocculation (p. 164), respectively. Electrophoresis permits more precise delineation of the plasma protein pattern, and is the only practical way in which the presence of qualitatively abnormal proteins can be demonstrated unequivocally. The clinical usefulness of this approach has been enhanced enormously by the introduction of the technic of paper electrophoresis, which has brought this valuable procedure within the scope of the routine clinical biochemistry laboratory.

Fibrinogen. The plasma fibrinogen (normal 200–400 mg. per 100 cc.) is not significantly altered in disturbances of protein metabolism per se.

INCREASED PLASMA FIBRINOGEN. Rouleaux formation and erythrocyte sedimentation in vitro are influenced strikingly by the plasma fibrinogen concentration. Increase in this factor is the most important cause of the increased sedimentation rate in infections and other diseases, e.g., multiple myeloma, nephrosis. The alpha- and beta-globulins are next in order of activity. Although the sedimentation-accelerating effect of gamma-globulins is less marked, they usually contribute more to the production of this phenomenon in disease because the increase in their concentration generally far exceeds that of the other globulins. Albumin exerts an opposing effect, tending to diminish erythrocyte sedimentation.

(1) Conditions causing slight hepatic injury (hepatitis) and tissue destruction (inflammatory) are frequently associated with moderate increases in plasma fibrinogen.

(2) Most acute infections (except typhoid fever), septicemias, bacteremias, and particularly pneumococcic pneumonia, in which condition values up to 1000 mg. per 100 cc. may be observed.

(3) Pregnancy and menstruation (400–500 mg. per 100 cc.).

(4) Following x-ray irradiation.

(5) Focal infection (sinusitis, tonsillitis, cholecystitis).

(6) Nephrosis. In this condition the plasma fibrinogen may rise to 1000 mg. per 100 cc., the mechanism being unknown.

(7) Increased fibrinogen values have been reported in multiple myeloma, lymphopathia venereum, and certain cases of cirrhosis.

Decreased Plasma Fibrinogen. (1) Hepatic Insufficiency. Since fibrinogen is formed in the liver, conditions associated with diminution in hepatic function may result in a decreased concentration of plasma fibrinogen. This is particularly true of acute hepatic insufficiency such as is seen in severe arsphenamine hepatitis, chloroform, phosphorus, and carbon tetrachloride poisoning, and in acute yellow atrophy of the liver, in which condition extremely low values may be encountered. It is likewise decreased in some, but not in all cases of cirrhosis of the liver.

(2) Typhoid Fever. The plasma fibrinogen is usually moderately diminished in typhoid fever, which is one of the very few infectious diseases in which this condition is observed.

(3) Temporarily, following severe hemorrhage.

(4) Cachectic conditions, notably malignancy.

(5) Constitutional Fibrinogenopenia. In this condition there may be delayed coagulation of blood or profuse bleeding after slight trauma, due to an inadequate amount of fibrinogen.

(6) Subnormal values have been observed occasionally in pernicious anemia, pellagra, scurvy, myelogenous leukemia, and other disorders of the bone marrow.

(7) Afibrinogenemia in Pregnancy. Uncontrollable hemorrhage associated with incoagulability of the blood is one of the most serious complications of labor. It may occur in long-standing intrauterine death, premature separation of the placenta, and in so-called "amniotic fluid embolism" following administration of pitocin. It has been suggested that this condition is due to entrance into the blood stream of a thromboplastin-like substance present in placental tissue and amniotic fluid, as a result of sharp increase in intrauterine pressure, with consequent intravascular clotting and defibrination of the blood.

Albumin. Increase in the concentration of albumin in the plasma is seldom encountered clinically. It may occur in dehydration with diminution in the water content of the plasma and consequent increased concentration of all nondiffusible components. Although theoretically possible, this condition is actually rarely observed, for in most instances in which dehydration is a prominent feature, such complicating factors as malnutrition, diarrhea, vomiting, etc. exert an influence which tends to decrease the plasma albumin concentration. However, total protein values of 10 to 12 gm./100 cc. due to this cause have been reported in patients with cholera, other forms of severe diarrhea, particularly in children, shock, burns, diabetic acidosis, Addison's disease, intestinal obstruction, intestinal fistula, pyloric obstruction, rigid restriction of fluid intake, heat exhaustion, and certain fulminating infections. In such cases, as indicated previously, the true nature of the situation can be revealed only by determination of the circulating plasma volume. The existence of an actual plasma albumin deficit is revealed at times by rapid restoration of extracellular fluid volume by therapeutic administration of salt solution (p. 288).

Decrease in plasma albumin (normal 3.6–4.5 gm./100 cc.) is of practical significance in several groups of clinical disorders, which are outlined below. One of the most important functions of albumin is in the maintenance of the colloid osmotic pressure of the blood plasma (p. 145) and, consequently, in the regulation of the distribution of water between the intravascular and the interstitial tissue compartments.

Edema is the most prominent feature of reduced plasma albumin concentration, the lowered colloid osmotic pressure tending to diminish the passage of water from the interstitial tissue spaces into the capillaries. The frequently associated increase in globulins is incapable of compensating effectively for the decrease in albumin because, their molecular size being much greater, they exert much less effect than albumin on osmotic pressure (p. 146). By direct measurement, the colloid osmotic pressure (C.O.P.) of normal serum or plasma has been found to be 280 to 470 mm. H_2O (average 360 mm.), with slightly lower values in normal pregnancy (average 300 mm.). Various formulas have been evolved for calculating the C.O.P. from the albumin and globulin concentrations; e.g., $C.O.P. = 45.2\ A + 18.8\ G$, where A is the albumin and G the globulin concentration in grams per 100 cc. However, such prediction equations are of no clinical significance.

There is a linear relation between the plasma specific gravity and total protein content, normal heparinized plasma (7 gm. protein/100 cc.) having a specific gravity of approximately 1.027 and an increase or decrease of 1 gm./100 cc. causing a rise or fall of 0.0029. Determination of plasma specific gravity has therefore been proposed as a rapid means of estimating the protein content, especially in dehydration and shock. Inasmuch as albumin and globulin have about the same effect on specific gravity, this procedure cannot reveal simultaneous changes in these factors in opposite directions.

Frequent attempts have been made to relate the development of edema to a certain level of hypoalbuminemia. It has been suggested, for example, that patients with nephritis develop frank edema (noncardiac) when the albumin falls below 2.5 ± 0.2 per cent ("edema level"). Experimentally, it has been found that edema appears when the albumin falls below 0.8 gm./100 cc. and disappears when it rises above 1 gm./100 cc. Similar statements have been made in relation to the occurrence of ascites in cirrhosis. It is stated, too, that edema usually appears when the plasma specific gravity falls below 1.023. However, no categorical statement can be made in this connection. Because of the operation of numerous other complicating factors, edema may be present at higher and absent at lower levels of plasma albumin, C.O.P., or specific gravity in different subjects or in the same subject at different times. These factors include abnormal variations in the size of the protein molecules, and in capillary permeability, capillary (i.e., venous) blood pressure, lymph flow, tissue tension, and sodium intake (p. 315).

As indicated elsewhere (p. 159), the plasma globulins are frequently, but not invariably increased in conditions in which the albumin concentration is low. This is not fundamentally a relationship of cause and effect (p. 159); more probably, the condition which underlies the depletion of albumin simultaneously stimulates the formation of certain globulins, e.g., antibodies (γ-globulins), lipoproteins (α- and β-globulins). As a result of these changes, the albumin : globulin ratio, normally about 1.5 : 1, is decreased or may be reversed. This factor is without clinical significance, since it may reflect a decrease in albumin, an increase in globulin, or a combination of both. The factors of clinical importance are the absolute levels of albumin and globulins, not their concentration ratio.

The causes of decrease in plasma albumin concentration, i.e., *hypoalbuminemia*, may be outlined as follows:

(1) LOSS OF ALBUMIN. This occurs most commonly, clinically, in nephritis and nephrosis with excessive and prolonged albuminuria. In chronic nephritis as much as 60 gm. of albumin may be eliminated daily in the urine and a daily loss of 4–5 gm. may eventually result in a gradual decrease in the level of plasma protein. The plasma protein concentration usually remains within normal limits in the early stages of acute glomerulonephritis. Van Slyke has found that cases of this condition that maintain plasma albumin values above 2.2 gm. per 100 cc. or total protein values above 5.5 gm. per 100 cc. have a better prognosis than those which do not. Of his cases in the group with higher albumin values, only 13 per cent progressed into the chronic or terminal stages of nephritis, in contrast to 72 per cent of the group with low albumin values. It would appear, therefore, that the plasma protein concentration is of prognostic significance during the acute stage of glomerulonephritis. It was also found that a low plasma albumin concentration, usually below 2.5 gm. per 100 cc., was the rule in the chronic active stage of glomerulonephritis. As the chronic active stage progressed into the terminal stage, there was a marked tendency for the plasma proteins to increase, normal values being obtained in about half the cases during the terminal stage of the disease. This may be due in part to decreasing albuminuria and in part to hemoconcentration.

Extremely low values may occur in chronic nephrosis, i.e., chronic active glomerulonephritis, falling at times to 0.2 gm. per 100 cc. Prolonged albuminuria in amyloid disease of the kidneys and congestive heart failure may contribute to the development of hypoproteinemia. In nephrosis particularly, the globulin and fibrinogen are commonly markedly increased, the former rising to as high as 6 gm. per 100 cc. and the latter to as high as 1 gm. Decrease in albumin, with or without a decrease in the total plasma protein concentration, occurs commonly in multiple myeloma; in some cases the globulin fraction is enormously increased, with consequent hyperproteinemia (p. 161).

The blood plasma is the source of the albumin present in the urine. Similar changes in albumin, without increase in globulin, have been produced experimentally by repeated plasmapheresis, with withdrawal of blood, separation of the plasma and reinjection of the corpuscular elements. It seems certain that the diminution in serum albumin which occurs in renal disease is not due entirely to loss of albumin in the urine. There appears to be a distinct nutritional defect which interferes with the ability of the organism to regenerate serum albumin. The importance of this factor is not sufficiently appreciated. It probably plays an important part in determining the development of hypoproteinemia in chronic glomerulonephritis. In the terminal stages of this condition the true extent of the serum albumin depletion may be masked by the development of a state of hemoconcentration, which may result from vomiting, anorexia, and the inability to conserve the salt and water stores of the body. A diminution in serum albumin may occur in acute glomerulonephritis, particularly in the transition between this stage and the chronic active stage of the disease. Here it appears to be referable to loss of albumin in the urine, malnutrition, and impairment of the ability to regenerate serum albumin.

In certain cases of ascites, the ascitic fluid containing a large amount of protein, depletion of the blood proteins may occur with changes similar to those observed in chronic nephritis with prolonged, excessive albuminuria. Edema may be present in such patients in the absence of renal or cardiac lesions. In advanced stages of chronic hepatic disease, including portal cirrhosis, there is also a reduction in total serum protein, the diminution occurring in the albumin fraction. In some cases, particularly in acute forms of liver disease, the serum albumin may be only moderately reduced and the serum globulin increased (p. 506). Such findings are obtained much more commonly in primary hepatocellular disorders than in obstructive jaundice. The decrease in serum albumin in hepatic disease has been attributed by some chiefly to coexisting malnutrition, and by others chiefly to interference with regeneration of serum albumin resulting from impairment of liver function. In cases in which ascites is present, the loss of albumin from the blood plasma into the ascitic fluid probably plays some part in contributing to the plasma protein depletion.

Large amounts of albumin may be lost from the plasma also in extensive burns, severe hemorrhage and polyserositis (Pick's disease). Hypoproteinemia has been observed in shock due to burns, intestinal obstruction, and generalized peritonitis, due presumably to loss of plasma from the circulation.

(2) Inadequate Supply of Protein. This may occur in states of inanition, as in advanced tuberculosis and malignancy. It is observed in gastrointestinal disorders accompanied by impaired protein digestion or absorption, prolonged vomiting, or diarrhea. Prominent among such conditions are peptic ulcer, gastrointestinal malignancy, pancreatic disease, intestinal fistula, short-circuiting operations on the intestine, chronic ulcerative colitis, tuberculous and other forms of chronic enteritis, steatorrhea (celiac disease, sprue), congestive heart failure with edema of the gastrointestinal mucous membrane, pellagra. This condition may also be associated with specific dietary deficiency characterized by low protein intake. This is well illustrated by the many instances of war edema, famine edema or nutritional edema which were observed during World War I and in inmates of prison camps in World War II. Edema has been induced at will in children suffering with chronic glomerulonephritis and nephrosis by varying the protein intake. When sufficient protein was administered to maintain a continuous positive nitrogen balance of +2 to +5 gm., edema did not develop. Inadequate protein feeding resulted in the prompt development of edema with diminution in the plasma albumin concentration.

(3) Impaired Synthesis. The importance of this factor is not sufficiently appreciated. It probably plays an important part in maintaining the low level of plasma albumin in chronic hepatic disease, particularly cirrhosis, and in chronic glomerulonephritis, various infections, severe anemias (e.g., uncontrolled pernicious anemia), cachectic states, and pregnancy and lactation. Observations in

experimental animals indicate that, under conditions of simultaneous hepatic damage and dietary protein restriction, the functional capacity of the liver, rather than the protein intake, is the limiting factor in determining the plasma protein concentration (p. 505).

(4) Excessive Protein Catabolism. Excessive breakdown of body protein, in conjunction with either inadequate supply or defective utilization, as in uncontrolled diabetes mellitus and severe thyrotoxicosis, may be accompanied by hypoproteinemia.

(5) Pregnancy and Lactation. Several observers have reported a steady decrease in plasma protein concentration, due to diminution in the albumin fraction, during the course of normal pregnancy. Actually, subnormal values are rarely observed under normal conditions. It is believed by some, however, that an aggravation of this natural tendency induced by dietary restriction of protein may lead to harmful consequences. Low values for plasma albumin and total protein have been reported in patients with toxemia of pregnancy, particularly eclampsia. The decrease in the concentration of plasma protein in normal pregnancy is attributed, in part at least, to the appreciably increased plasma hydration that occurs during that period (p. 299). However, it has been shown that not only the concentration but also the total amount of circulating plasma protein is diminished. This diminution is associated with distinct impairment of the ability to regenerate serum protein. Some believe that synthesis of body proteins in the fetus constitutes a drain upon the maternal organism which is of primary importance in causing the lowered serum protein concentration characteristic of pregnancy. Similar findings have been obtained during lactation.

(6) Plasma Dilution. Hypoproteinemia may result from sudden dilution of the plasma which follows an acute, massive hemorrhage. A similar phenomenon may accompany sudden recovery from severe dehydration in malnourished subjects, as in diabetic coma and protracted vomiting or diarrhea (especially in children). In the dehydrated state, the actual plasma protein deficit may be masked by hemoconcentration (loss of plasma water). When the latter is corrected by administration of large quantities of solutions of sodium salts (chloride, bicarbonate, lactate, etc.), the plasma protein concentration may fall to a level sufficiently low to produce edema.

The frequently diminished plasma protein concentration in congestive heart failure is due in part to increased plasma volume (water). As indicated above, it is also contributed to in such cases by prolonged albuminuria, decreased protein intake, impaired digestion, diminished absorption from the edematous intestinal mucosa, and impaired synthesis (hepatic malfunction).

Globulins. The several approaches to the investigation of possible abnormalities in the plasma globulins are indicated elsewhere (pp. 143, 163). It will be recalled that the classical Howe fractionation procedure is unreliable, yielding erroneously high values for albumin and low values for total globulins (Table 3, p. 143). The fact must be kept in mind that the globulin fraction, as determined by chemical methods, is an extremely heterogeneous group of substances. Over thirty different components have been characterized and identified, some of which are indicated in Figure 19 (p. 146). Inasmuch as many if not all of these are functionally and, presumably, structurally distinct, it is logical to assume that independent variations may occur in single components, or in small groups of related components, in response to various types of stimuli or as a result of disease. Theoretically, at least, different globulin fractions may vary in opposite directions, with no significant change in the total globulin concentration. Such abnormalities may be revealed only by more refined analytical procedures, such as pseudo- and euglobulin fractionation or, more precisely, by electrophoresis. Useful information in this connection may be obtained also by application of so-called "globulin reactions" (p. 163), such as the zinc sulfate turbidity, thymol turbidity, and cephalin-cholesterol flocculation tests.

Decrease in plasma globulins (normal, 2.1–4.2 gm./100 cc.) is not encountered frequently clinically. Low concentrations (below 1 gm./100 cc.) may occur, associated with hypoalbuminemia, in patients with steatorrhea and advanced malnutrition (e.g., celiac disease, sprue). It has also been observed, occasionally, in protein starvation, e.g., in inmates of prison camps in World War II, and in experimental animals subjected to plasmapheresis.

A condition, called "agammaglobulinemia," has been described, in which there is an absence (or extremely low levels) of γ-globulin in the serum and an inability to respond to artificial immunization with the production of circulating antibodies. Other features of this syndrome include: (1) absence of isohemagglutinins; (2) history of recurrent bacterial infections; (3) failure of prolonged antibiotic therapy to furnish protection; (4) satisfactory response to protective injections of gamma globulin. It is apparently more common in children, and the question of a congenital defect of the antibody-forming tissues has been raised. However, its occurrence also in adults suggests that the condition may be acquired. The cases reported thus far in children have been in males.

The frequent association of hyperglobulinemia with hypoalbuminemia is referred to elsewhere (p. 155). Certain generalizations may be made concerning the conditions in which the various globulin fractions are increased.

ALPHA-GLOBULINS (PSEUDOGLOBULIN FRACTION). These increase rather consistently in acute febrile disorders and conditions accompanied by inflammatory or destructive lesions, e.g., cirrhosis, nephrosis. The underlying mechanism is not known. An increase occurs also in many cases of multiple myeloma. The α-glycoproteins are included in this fraction, and also certain α-lipoproteins (e.g., increased in essential hyperlipemia).

BETA-GLOBULINS (EUGLOBULIN FRACTION). Increase in this group of globulins is observed commonly in association with increase in plasma lipids, e.g., nephrosis, hypothyroidism, certain liver diseases, hypercholesterolemic xanthomatosis, etc. The β-lipoproteins (p. 112) are included in this fraction, which contains also certain antibodies. An increase occurs, therefore, in certain infectious diseases, and also in some cases of multiple myeloma.

GAMMA-GLOBULINS (EUGLOBULIN FRACTION). This is the fraction that is increased most frequently and, usually, most strikingly. In some cases it may comprise as much as 50 per cent or more of the total plasma proteins. In man, most but not all antibodies are γ-globulins and, therefore, this fraction would be expected to increase in infectious and other diseases evoking an immunological response. The most marked elevations occur usually in (*a*) viral and protozoal diseases, (*b*) extensive tissue destruction or suppuration, (*c*) extensive granulomatous proliferation, and (*d*) certain other conditions, such as multiple myeloma, hepatic cirrhosis, hepatic necrosis, and hypothyroidism. This increase in γ-globulin is the cause of the anticomplementary reactions commonly obtained in serological tests for syphilis with serum from patients with such diseases as kala-azar, lymphopathia venereum, liver disease, and multiple myeloma.

GLYCOPROTEINS. These are conjugated proteins containing carbohydrate (glucose, galactose, hexosamines). They occur in certain hormones, viz., gonadotrophins, in the blood-group substances, and in the "ground substance" of various tissues, e.g., in hyaluronic acid. They are present in highest concentration in α- and β-globulin fractions, but are also associated with γ-globulins and, to a lesser extent, with albumin. Interest has been focused on these substances particularly because of their relation to the so-called "collagen diseases."

They have been found to be increased in the plasma in many acute and chronic inflammations, and in conditions accompanied by tissue destruction. Among these are: pneumonia, rheumatic fever, rheumatoid arthritis, periarteritis nodosa, inflammatory dermatoses (e.g., disseminated lupus erythematosus), myocardial infarction, carcinomatosis, leukemia, Hodgkin's disease, chronic infections, and trauma. In several of the inflammatory conditions, e.g., acute disseminated lupus, the concentration of glycoproteins falls after administration of ACTH or cortisone. In the majority of these the increase is in the α-glycoprotein fraction (i.e., α-globulin). β-Glycoproteins are increased in conditions accompanied by hyperlipemia, especially renal and hepatic diseases, but also diabetes mellitus. An increase has been reported also in intestinal lipodystrophy. The normal range of hexosamines in the plasma is stated to be 50 to 90 mg./100 cc.

CRYOGLOBULINS. This term is applied to proteins which precipitate from abnormal sera spontaneously, usually only in the cold (7–11° C.), and which usually, but not invariably, redissolve at room temperatures. They resemble γ-globulins. They have been observed in the following conditions: multiple myeloma (usually they are not Bence Jones proteins), kala-azar, periarteritis nodosa and other disorders accompanied by eosinophilia, chronic glomerulonephritis with "acropurpura," and chronic infectious arthritis. In some cases these proteins may undergo gelation or precipitation on standing at room temperatures.

The association of purpuric manifestations with the presence of these globulins has given rise to the term, "purpura cryoglobulinemica." It is assumed that precipitation of cryoglobulins in small vessels of the skin on cooling causes Raynaud-like symptoms, purpura and, in severe cases, ulceration and necrosis of the skin.

PYROGLOBULINS. This term has been applied to plasma proteins that gel on exposure to moderate elevation of temperature (e.g., 55° C.); they may not redissolve on returning to room temperature, contrasting with cryoglobulins in this respect. They have been observed in multiple myeloma, being detected usually in the course of inactivating the serum complement in performing serological tests for syphilis.

Bence Jones Protein. This term is applied to a group of proteins with a molecular weight of approximately 37,000, the physical state of which is altered in vitro by temperature changes, usually precipitating from solution at 40 to 60° C. and redissolving, partially or completely, at 95 to 100° C. They appear most commonly in the urine (p. 177), and have been demonstrated in the blood in occasional cases of multiple myeloma, but perhaps occur in amounts too small to be demonstrable in most if not all patients with Bence Jones proteinuria.

Conditions in which hyperglobulinemia occurs rather characteristically may be outlined as follows:

Acute and Chronic Bacterial Infections. These include pneumonias, subacute bacterial endocarditis, tuberculosis, and leprosy. Total protein values as high as 10 gm./100 cc. have been reported in subacute bacterial endocarditis, with globulin values as high as 8 gm./100 cc. There is evidence that the maintenance of a high plasma globulin concentration is related to the development of amyloid disease in chronic suppurative diseases.

The globulin increase in these conditions is mainly in the α- and γ-globulins, but occasionally also in the β-globulins. The plasma albumin is usually decreased simultaneously. The degree of hyperglobulinemia does not appear to be related to the height of the fever nor to the severity of tissue inflammation or destruction.

Viral and Parasitic Diseases. With the exception of multiple myeloma, the largest globulin increases are usually seen in certain diseases in this category, e.g., infectious hepatitis, kala-azar, typhus, lymphopathia venereum. In the latter condition, total protein values over 11 gm./100 cc. have been reported. Hyperglobulinemia, usually of lesser degree, may occur also in syphilis, malaria, trypanosomiasis, filariasis, schistosomiasis, and histoplasmosis. The participation of cells of the reticuloendothelial system in the tissue response in certain of these disorders appears to have some relation to the production of the hyperglobulinemia. The increase is mainly in γ-globulins, but also in the α-globulin fraction.

Multiple Myeloma. Hyperglobulinemia has been observed in up to 75 per cent of cases of multiple myeloma, of a degree sufficient to produce hyperproteinemia (up to 16 gm./100 cc.) in about 50 per cent of cases. The increase is usually mainly in the γ-globulin (euglobulin) fraction, but no consistent pattern has been observed. Increases may occur in both α- and β-globulins, and in some instances a component has been described which apparently satisfies the criteria for Bence Jones protein. Observations in this disease suggest the hypothesis that the globulin-synthesizing mechanism is fundamentally disturbed, with the excessive production of a homogeneous component, differing in different cases, that is normally present only in low concentration. There is no consistent relationship between the plasma globulin pattern and the presence of Bence Jones proteinuria (p. 178).

Hepatic Disease. Changes in plasma proteins in various types of liver disease are discussed in detail elsewhere (p. 505ff.). Increase in γ-globulin in infectious (viral) hepatitis is referred to above. Hyperglobulinemia occurs rather characteristically also in other forms of acute and chronic liver damage, e.g., acute and subacute hepatic necrosis (e.g., carbon tetrachloride poisoning, idiopathic hepatic necrosis), kwashiorkor, and cirrhosis. Extremely high values (over 6 gm./100 cc.) have been reported in hepatic necrosis and in cirrhosis, at times adequate to produce an increase in total protein despite the coexisting hypoalbuminemia. The increase is usually mainly in γ-globulin, and has been attributed by some to production of antibodies to an altered (i.e., foreign) liver protein. Variable increases may occur also in the α- and β-globulins, the latter usually in those cases in which hyperlipemia is present.

KIDNEY DISEASE. The most striking changes in the plasma globulin pattern occur in the nephrotic stage of chronic glomerulonephritis. These are invariably associated with hypoalbuminemia, and consist in an increase in total globulin concentration, due usually mainly to increases in α_2- and β-globulins. Fibrinogen is rather consistently increased and the γ-globulin fraction decreased. The rise in β-globulins is related to the coexisting hyperlipemia and elevated concentration of β-lipoproteins and S_f 12–20 molecular classes (p. 112). Similar but generally less marked changes may occur in amyloid nephrosis. Slight to moderate increases in one or all of the three globulin fractions may occur, inconsistently, in acute glomerulonephritis and in stages of chronic glomerulonephritis other than the nephrotic.

SARCOIDOSIS. Hyperglobulinemia occurs rather consistently during active stages of this condition, frequently of marked degree, i.e., above 5 gm./100 cc., usually in association with hypoalbuminemia. The major increment is in the γ-globulin fraction, although the α-globulins may be elevated somewhat. The total protein concentration may be increased. It has been suggested that these changes may aid in differentiating between sarcoidosis and tuberculosis; in the latter condition, hyperproteinemia and marked hyperglobulinemia are not the rule (except with extensive lymphatic involvement), although increases in α- and γ-globulins may occur. However, the abnormalities in sarcoidosis are not always striking, especially in less active forms of the disease.

MISCELLANEOUS. Increase in β-globulins has been found to occur in most cases of hypothyroidism, usually associated with hyperlipemia (p. 117); the γ-globulins are apparently elevated in about 50 per cent of cases.

An abnormal "α_x-globulin" has been reported in hemophilia, and also in over 50 per cent of both male and female members of hemophilic families who are not themselves bleeders. The occurrence of this abnormality has been related to the presence of a low rate of utilization of prothrombin exhibited by the plasma of these individuals.

Marked increases in γ-globulins have been observed in second and third degree burns involving more than 25 per cent of the body surface.

Increase in α- and γ-globulins occurs in many patients with rheumatic fever and rheumatoid arthritis. The rise in γ-globulins appears to parallel the increasing titers of antistreptolysin and streptococcal agglutinating factor, respectively, in these conditions, but cannot be attributed entirely to these factors.

Increase in α-globulin has been reported in patients with leukemia and carcinomatosis. It may be related to the occurrence of intercurrent infections or to extensive degenerative changes in malignant lesions rather than to the primary processes.

Globulin Reactions

Certain qualitative reactions that yield abnormal results in the presence of abnormalities in one or more plasma globulin fractions have been found to be very useful clinically. Several of these have been described, the most widely employed currently being the thymol turbidity, cephalin-cholesterol flocculation, and zinc sulfate turbidity tests.

Zinc Sulfate Turbidity. This procedure is based upon the fact that the solubility of the plasma proteins is decreased upon dilution with solutions of low ionic strength; under certain conditions the more insoluble globulins will precipitate, with the development of measurable turbidity. With appropriate concentrations of such heavy metals as zinc or copper, it is possible to produce a situation in which there is minimal precipitation from normal serum and maximal precipitation from sera with increased concentrations of γ-globulin. Turbidity measurements are made by comparison with a standard barium sulfate solution. The normal range is 2 to 8 units.

As is true of other globulin reactions, this procedure is applied most extensively in the study of patients with hepatic or biliary tract disease, and is considered in more detail elsewhere (p. 510). Its chief advantage over the thymol and cephalin tests lies in the fact that it appears to reflect quantitative changes in a single component of the plasma, viz., gamma-globulin, whereas the other reactions are influenced by associated changes in albumin and lipid (p. 164).

The zinc sulfate turbidity test has also been used to study the development of antibodies in patients with scarlet fever and rheumatic fever. Increased turbidity has been found to parallel rises in antistreptolysin and antistreptokinase titers in these diseases.

Formol-Gel Reaction. The addition of formaldehyde to normal serum, under standardized conditions, results in no change in viscosity or translucency. Positive reactions (abnormal) are indicated by increased viscosity (gelation), with or without increased opacity. A positive reaction is commonly obtained in conditions accompanied by significant hyperglobulinemia, but may also occur in other conditions, the exact mechanism of its production being still questionable.

Positive reactions have been reported in multiple myeloma, subacute bacterial endocarditis, sarcoid, hepatic cirrhosis and other forms of hepatic parenchymal disease, nephrosis, malaria, trypanosomiasis, kala-azar, schistosomiasis, and a variety of other acute and chronic infectious diseases. A strongly positive reaction in rheumatic fever has been reported to be suggestive of the presence of rheumatic carditis.

Colloidal Gold Curve. A paretic type of colloidal gold curve has been obtained with the blood serum of a considerable proportion of patients with various forms of liver disease (cirrhosis, necrosis, hepatitis, malignancy), due apparently to some qualitative or quantitative change in the euglobulin or gamma-globulin fraction.

CO_2 Saturation Test. In the presence of globulin concentrations over 4 to 5 per cent, the addition of distilled water to the serum causes clouding due to flocculation of euglobulin. This reaction can be accelerated by saturating the diluted serum with CO_2 and by lowering the pH toward the isoelectric point of globulin. Positive (abnormal flocculation) reactions have been obtained in conditions accompanied by serum globulin concentrations above 4 gm. per 100 cc. and in some cases with normal serum globulin concentrations. In the latter, the reaction may be dependent upon an increase in the euglobulin fraction. Results obtained with this procedure are approximately the same as with the Takata-Ara and formol-gel reactions.

Cephalin-Cholesterol Flocculation Test. This test is based upon the observation that when diluted serum with an abnormally high globulin content is added to an emulsion of a cephalin-cholesterol mixture, the latter substances (plus globulin) precipitate out (flocculation) and the solution is clarified (opalescence decreases). The degree of flocculation and clarification is recorded as zero to 4 plus. This test gives positive results (flocculation) in a large proportion of cases of active hepatocellular damage and is widely used in the study of patients with jaundice (p. 510). Positive findings are also obtained in most of the other conditions mentioned above in which there is an increase in globulin, especially if the albumin is decreased. The production of a positive reaction in disease has been attributed to the existence of one or more of the following conditions:

(1) Increase of gamma-globulin in such quantity that the normal components of the serum albumin fraction are unable to inhibit the reaction.

(2) Decrease in serum albumin concentration below that capable of inhibiting the reaction.

(3) Decrease in flocculation-inhibiting properties of the serum albumin fraction due to its chemical modification.

Positive reactions may be obtained with normal serum (*a*) after standing at ice-box temperatures for several months, (*b*) after heating to 56° C. for thirty minutes or (*c*) if the reaction mixture is exposed to light during the test period.

Thymol Turbidity Test. This test is based upon the observation that addition of thymol solution to certain sera with abnormally high globulin contents results in marked turbidity of the solution (thymol-globulin-lipid complex), whereas little or no such reaction occurs with normal serum. The degree of turbidity (expressed in units) may be measured by comparison against a turbidity standard, e.g., barium sulfate. The normal range is 1 to 4 units, usually read at 30 minutes. After longer periods, flocculation may occur, as in the cephalin-cholesterol test. This observation is usually made at 18 hours, reactions greater than 1 + being regarded as abnormal. The turbidity and flocculation reactions do not always parallel one another. The thymol turbidity test clinically gives results that generally parallel those obtained with the cephalin-cholesterol flocculation test (above). Certain differences have been observed, however, which indicate points of difference in the fundamental mechanism involved. Cephalin-cholesterol flocculation is obtained with the serum of normal laboratory animals, including dogs and rabbits, which gives a negative thymol turbidity test. On the other hand, the latter test gives an abnormal response with certain lipemic sera, as in diabetes mellitus and nephrosis, whereas the former does not. Abnormally high values are obtained also with the serum of normal subjects after a high-fat meal, the degree of abnormality roughly paralleling the increment in plasma lipids. This test should be performed, therefore, on serum collected after an overnight fast (10–12 hours). Whereas purified gamma-globulin produces and purified serum albumin fractions protect against cephalin-cholesterol flocculation, these fractions have little effect upon the thymol turbidity reaction, whether derived from either normal or abnormal sera. It has been suggested that the basic condition necessary for the production of a positive thymol turbidity test consists in the presence of an abnormally high β-globulin (β-lipoprotein) fraction.

Other Globulin Reactions. The Weltmann reaction is based upon the fact that a 1:50 dilution of serum does not coagulate on boiling, but will do so when a minimal amount of calcium chloride is added. With normal serum the concentration of added calcium chloride must be at least 0.35:1000, while coagulation may occur with concentrations as low as 0.2:1000 in serum from patients with conditions mentioned in connection with the reactions discussed above. This phenomenon apparently depends upon the presence of an increase in euglobulin, mainly γ-globulin, as does the magnesium flocculation reaction (Bauer).

The Takata-Ara test depends upon abnormal precipitation in serial dilutions of serum on addition of a dilute solution of mercuric chloride in the presence of small amounts of sodium carbonate. This phenomenon is apparently dependent upon abnormalities in the ratio of albumin to globulin, particularly the γ-globulins. Positive results have been obtained in a variety of diseases, including cirrhosis, nephrosis, multiple myeloma, sarcoidosis, lymphopathia venereum, pneumonia, syphilis, tuberculosis, etc.

Protein Diagnostic Tests for Cancer

A number of diagnostic tests for cancer have been proposed that depend upon some abnormality in the plasma proteins. The following have attracted considerable attention:

(*a*) Defective thermal coagulation of serum proteins. This is a measure of the lowest concentration in which the serum proteins are coagulable by heat (higher than normal in cancer).

(*b*) Iodoacetate Index. This is a quantitative expression relating the inhibition of coagulation by iodoacetate to the concentration of serum proteins (coagulation defect in cancer).

(*c*) Serum protein precipitation by acidified sodium orthovanadate. In cancer, precipitation begins in mixtures containing more acid and less vanadate.

None of these tests is in any way specific for cancer, inasmuch as they depend upon protein changes that occur in a number of diseases. The numerous false positive and false negative results make the application of such procedures in the diagnosis of cancer unreliable and hazardous.

NONPROTEIN NITROGEN OF BLOOD

Alterations in the total nonprotein nitrogen content of the blood depend upon variations in the concentrations of its constituent elements, which will be considered individually (see also Renal Function, p. 606).

Urea Nitrogen (normal 9–17 mg. per 100 cc.). INCREASED UREA N (see Renal Function, pp. 606–611). Increase in blood urea N may be due to (*a*) decreased renal excretion, (*b*) absorption from the intestine of excessive amounts of products of protein digestion, or (*c*) excessive protein catabolism, or to a combination of these factors. Elevation of blood NPN or urea N due primarily to factors other than renal or urinary tract disease has been termed "extrarenal azotemia." Decreased renal excretion of urea may be due to (*a*) organic disease of the kidneys, with destruction of a considerable portion of functioning renal tissue, or (*b*) abnormality of the mechanism of glomerular filtration, resulting in significant decrease in the effective filtration pressure (p. 588) (e.g., marked hypotension, increased capsular pressure, hemoconcentration, hyperproteinemia). The most common cause for increased blood urea N (and NPN) is inadequate excretion, due usually to kidney disease, urinary obstruction, or extreme hypotension and oliguria.

(1) *Glomerulonephritis.* This is a common cause for abnormally high blood urea N concentrations, evidence of renal functional impairment in acute and chronic glomerulonephritis. In the terminal state and during acute exacerbations of chronic nephritis, values as high as 200 mg. or more per 100 cc. may be obtained (p. 608).

(2) *Other Kidney Diseases.* These include (*a*) conditions in which there is extensive destruction or inflammation of the kidneys, as in renal tuberculosis, pyelonephritis, advanced nephrosclerosis, renal cortical necrosis, malignancy, suppuration, and chronic gout; (*b*) renal conditions accompanied by marked oliguria or anuria, as bichloride of mercury poisoning, postoperative urinary suppression, lower nephron nephrosis, and advanced myocardial failure with passive congestion of the kidneys; (*c*) congenital renal lesions, as hypogenesis or hypoplasia, and polycystic disease of the kidneys; (*d*) conditions in which the uriniferous tubules are blocked by precipitation of some substance which interferes with urine excretion. Among these are multiple myeloma, amyloid disease of the kidneys, hemoglobinuria, as after transfusion with incompatible blood, and sulfonamide therapy.

(3) *Urinary Tract Obstruction.* Enlarged prostate, urinary lithiasis, malignant obstruction of the ureters from extension of tumors of the bladder or uterus, bowel, retroperitoneal lymph nodes.

(4) *Hepatic and Biliary Tract Disease.* High NPN and urea N values are encountered at times in patients with hepatic disease. This occurs most commonly in terminal stages of acute or chronic liver disorders, after operation on the bile passages, particularly after decompression of obstructed bile ducts, and in traumatic necrosis of the liver. The mechanism operating in such cases to produce the so-called "hepatorenal syndrome" is not clearly understood.

(5) *Shock and Hemoconcentration.* Hemoconcentration resulting from loss of

plasma water by prolonged vomiting, diarrhea or sweating may result in elevation of blood urea, especially if accompanied by severe hypotension, as in the shock syndrome. These phenomena prevent adequate glomerular filtration by diminishing the effective glomerular filtration pressure and also result in extreme oliguria by prerenal deviation of water. In this category may be placed severe burns, high intestinal obstruction, pyloric obstruction, cholera, typhoid fever, infantile diarrhea, dysentery, Addison's disease, pancreatic fistula, peritonitis.

A peculiar form of delayed renal failure ("crush syndrome") has been observed following crushing injuries to large masses of muscle and characterized morphologically by necrosis of the renal tubular epithelium (lower nephron nephrosis). This has been variously ascribed to renal anoxia (ischemia), and to the toxic effects of methemoglobin, metamyoglobin and myoglobin. The mechanism is unknown (p. 610).

There is some evidence that the azotemia that follows extensive burns consists chiefly in an increase in amino N and undetermined N, the rise in the latter paralleling the severity of the clinical state. In shock (p. 321), the increase in blood amino N is due to an increase in protein catabolism in the anoxic tissues and diminished deamination of amino acids in the anoxic liver.

(6) *Excessive Protein Catabolism.* Slight or moderate increase in blood urea may occur in severe toxic and febrile conditions in which tissue protein catabolism is accelerated and in which there is also some degree of renal functional impairment. This factor may contribute to the increase in blood urea in many of the conditions mentioned above (acute intestinal obstruction, severe infections and burns), and to the increased blood amino N in shock (p. 321).

(7) *Miscellaneous.* Nitrogen retention may occur as a consequence of renal failure, usually as a terminal event, in patients with hyperparathyroidism (p. 234). This apparently depends in part upon extensive calcification of the renal tubular epithelium or upon the formation of urinary calculi; in acute hyperparathyroidism, hemoconcentration and impaired blood flow through the kidneys may contribute to the development of renal functional insufficiency.

Nitrogen retention is observed not infrequently in diabetic coma. The mechanism of its production is not clearly understood. However, dehydration, hemoconcentration, excessive protein catabolism, and perhaps diminished glomerular filtration due to intercapillary glomerulosclerosis and other forms of renal damage, may be of importance in this connection.

The blood urea may rise soon after hemorrhage into the gastrointestinal tract (peptic ulcer, carcinoma of the stomach, esophageal varices in cirrhosis of the liver, and so on) due probably to absorption of excessive amounts of products of protein digestion (from globin and albumin) and inadequate renal excretion.

DECREASED UREA N. (1) *Acute Hepatic Insufficiency.* As urea is normally formed in the liver and, presumably, through hepatic functional activity, it naturally follows that a decrease in blood urea may be expected in conditions associated with acute hepatic insufficiency. Subnormal values (5–10 mg. per 100 cc.) have been observed in acute yellow atrophy of the liver, acute toxic hepatic necrosis due to phosphorus, arsphenamine, chloroform and carbon tetrachloride poisoning, eclampsia, and in acute hepatic insufficiency following operative procedures upon the biliary tract. Such findings are rare in chronic hepatic disease such as cirrhosis, passive congestion or malignancy, because of the large functional reserve capacity and enormous regenerative power of the liver.

(2) *Normal Pregnancy.* The blood nonprotein nitrogen decreases during the first six months of pregnancy to levels of 20 to 25 mg. per 100 cc., increasing subsequently to reach an average of 25 to 30 mg. per 100 cc. at term. The urea nitrogen may fall as low as 5 mg. per 100 cc. after six months, these low levels being maintained until the eighth or ninth month, when it begins to rise, reaching values of 7 to 9 mg. per 100 cc. at term.

Uric Acid. Abnormalities in blood uric acid are discussed elsewhere (nucleic acid metabolism, p. 199).

Creatinine (normal, 1–2 mg. per 100 cc.). An increased concentration of creatinine may occur in any condition in which blood urea is increased, but usually only after the latter has risen to comparatively high levels. This is due in part perhaps to extrarenal causes, particularly the solely endogenous origin of creatinine as contrasted to the largely exogenous origin of urea, and also to the fact that the normal creatinine clearance is considerably higher than the urea clearance (pp. 589, 599).

(1) NEPHRITIS (see Renal Function, p. 606). When renal functional impairment is due to chronic nephritis, creatinine retention is of decidedly serious prognostic import, since the nature of the anatomic lesion renders functional improvement impossible. In chronic nephritis with uremia, figures as high as 35 mg. per 100 cc. may be obtained. In this condition, values above 5 mg. per 100 cc. usually indicate a hopeless prognosis, with a relatively short expectation of life. In acute nephritis, however, and in acute exacerbations of chronic nephritis, extremely high concentrations of creatinine may be found, which, with subsidence of the acute renal lesion, may return to normal (see Renal Function, p. 607).

(2) URINARY OBSTRUCTION. Prostatic and bilateral ureteral obstruction may, if the degree of back pressure rises sufficiently, be associated with creatinine, as well as urea and uric acid retention. Similar findings may be observed in unilateral ureteral calculus with reflex anuria. Under such circumstances, the process being purely mechanical in the absence of renal damage, the degree of elevation of blood creatinine is roughly proportional to that of urea; this is in striking contrast to the findings in chronic nephritis, in which condition high urea values may be observed in association with normal creatinine figures. In urinary obstruction, as in acute nephritis, in the absence of irreparable renal damage, extremely high creatinine values (15–30 mg. per 100 cc.) may return to the normal level following relief of the obstruction.

(3) URINARY SUPPRESSION (see Urea, p. 166).

(4) CARDIAC DECOMPENSATION (see Urea, p. 166).

(5) INTESTINAL OBSTRUCTION (see Urea, p. 166).

Amino Acid Nitrogen (normal, 5–8 mg. per 100 cc.). Increase in the amino acid content of the blood may occur in the following conditions:

(1) Hepatic Insufficiency. Amino acids normally undergo deamination in the liver with the consequent formation of urea. When the urea-forming or deaminizing function of the liver is impaired the concentration of amino acids in the blood increases and that of urea diminishes. The normal ratio of urea nitrogen to amino acid nitrogen is approximately 2 to 1. This ratio is, therefore, decreased in such conditions as acute yellow atrophy (acute toxic necrosis) of the liver, phosphorus, arsenic, chloroform and carbon tetrachloride poisoning and, less frequently, in acute hepatitis. Figures as high as 200 mg. per 100 cc. have been reported in cases of acute yellow atrophy of the liver but the usual range is 10 to 15 mg. per 100 cc., values above 30 mg. being infrequently observed. The increase in amino acid nitrogen is due partly to extensive autolysis of hepatic tissue. High values are not ordinarily found in chronic hepatic disease such as cirrhosis, chronic obstructive jaundice, hepatic syphilis, or malignancy because of the large functional reserve and marked regenerative power of the liver.

(2) Eclampsia. Figures of 6 to 12 mg. per 100 cc. are at times observed in eclampsia, due presumably to the hepatic lesions present in that condition.

(3) Interference with Elimination. The blood amino acid nitrogen may be found to be increased slightly in some cases of advanced nephritis with marked nitrogen retention and also in urinary obstruction or suppression. High values are, however, rare under these circumstances, unless there is an associated condition of hepatic insufficiency. In most cases of nephritis with high blood nitrogen values the amino acid content of the blood is within normal limits.

(4) Miscellaneous. An increase may occur occasionally in myeloid leukemia and, rarely, in diabetes mellitus, acute infections, congestive heart failure, hyperthyroidism, and severe anemia.

Subnormal levels have been reported in pneumococcic pneumonia and after administration of growth hormone, insulin, and androgens (p. 140).

Ammonia. An increase in blood ammonia has been observed in certain patients with severe liver damage with impending "hepatic coma." Although this relationship has not been shown to be consistent, it is believed by some to be important (p. 505). This increase is presumably due to inadequate utilization of ammonia by the functionally damaged liver (p. 504).

Undetermined or Residual Nitrogen (normal, 5–18 mg. per 100 cc.). The undetermined or "residual" nitrogen is contained chiefly in the corpuscles and is believed by some to be in the form of hippuric acid, nucleotides and histones. In some cases of eclampsia and advanced chronic nephritis with nitrogen retention and uremia the undetermined nitrogen fraction may be increased much more, proportionately, than the known nitrogenous elements. Some investigators believe that some component of this fraction may be responsible for the toxic manifestations of eclampsia and uremia. In some instances, however, no increase is demonstrable. A slight increase may occur in normal pregnancy.

Total Nonprotein Nitrogen (normal, 25–35 mg. per 100 cc. See Renal Function, pp. 606–611). An increase in the concentration of total nonprotein nitrogen occurs particularly in those conditions in which the blood urea content is increased. Extremely high values (to 400 mg. per 100 cc.) have been observed in chronic nephritis, acute nephritis, urinary obstruction, and renal destruction (tuberculosis, pyonephrosis, polycystic kidneys, etc.). Slight elevations may be found in gout and in myelogenous leukemia, in which conditions the uric acid concentration is increased independently of urea and creatinine. Normal values are usually present in eclampsia.

ABNORMAL URINARY NITROGEN

Protein in Urine

Under normal conditions very little or no protein is eliminated in the urine. The glomerular capillaries of the kidney may be collectively regarded as a semipermeable membrane, existing as a barrier between the blood plasma and the urine, allowing the passage of water and most crystalloids, but being practically impermeable to colloids. By utilizing extremely delicate reagents such as an acid alcoholic solution of phosphotungstic acid (Tsuchiya reagent) and by Kjeldahl determinations, small amounts of protein may be demonstrated in the urine of many normal individuals. Addis has placed the upper limit of normal variation in the neighborhood of 30 mg. of protein for the twelve-hour-day period but values as high as 75 mg. daily may be normal. This consists almost entirely of albumin with a small proportion of globulin; these are in all probability derived from the plasma proteins. This normal proteinuria cannot be detected by the qualitative methods most commonly employed clinically.

The Nature and Origin of Urinary Proteins. The term ''albuminuria'' has become so deeply rooted in medical literature that it seems advisable to point out the fact that proteins other than albumin may be and commonly are present in the urine. In fact, albumin seldom appears in the urine in any quantity without the simultaneous appearance of some other protein, notably globulin. Consequently the term ''proteinuria'' is more exact and more desirable than ''albuminuria.'' Because of the universal acceptance of the latter term, however, it will be employed throughout the subsequent discussion to designate that group of protein substances which respond to the commonly employed qualitative and quantitative tests for albumin (heat and acetic acid, nitric acid, sulfosalicylic acid, and so on). Only on rare occasions is fibrinogen present in the urine in renal disease.

The bulk of accumulated evidence points to the fact that the greater part of the protein found in the urine in renal disease is derived from the plasma proteins, albumin, pseudoglobulin, euglobulin and fibrinogen. The proportion of each of these substances eliminated by the kidney in nephritis appears to be determined by the relative size and viscosity of their molecules. Therefore albumin, having by far the lowest molecular weight and viscosity, is excreted in largest amount and fibrinogen, possessing the largest molecule, is eliminated in smallest quantity, pseudoglobulin and euglobulin occupying intermediate positions in the order named.

The relative proportions of albumin and globulin present in the urine in various types of renal disease have been extensively investigated. Although the determination of the urinary albumin : globulin ratio has not proved to be of practical clinical significance, certain observations are of interest inasmuch as they illustrate to some extent the nature of the renal functional disturbance in the presence of different renal lesions. Extremely high urinary albumin : globulin ratios have been found in lipoid nephrosis, figures above 10 being commonly observed. In chronic glomerulonephritis the ratio is usually lower, the majority ranging from 3 to 5, with a few between 5 and 10. In acute nephritis the figures are usually low (4–6), rising as the acute process subsides. Low values are obtained in amyloid disease of the kidneys (0.5–3.5). Since the plasma albumin : globulin ratio is normally about 1.5 : 1 and since the passage of various protein molecules through capillary walls and other living membranes is dependent upon increased permeability of those membranes, it appears that in amyloid disease the permeability of the kidneys is so increased that the relatively large globulin molecule passes freely, resulting in a urinary albumin : globulin ratio which approaches that present at the time in the blood plasma. Likewise, in acute nephritis, glomerular capillary membrane permeability is so increased that globulin passes with relative ease; as the acute lesion subsides permeability diminishes and the ratio increases correspondingly. Finally, in nephrosis, membrane permeability being relatively low, only small amounts of globulin are eliminated whereas the comparatively small albumin molecule passes through in large amounts.

The preceding brief discussion of the mechanism of proteinuria is based upon the conception that the bulk of the urinary proteins consists of normal plasma proteins and that their appearance in the urine is determined by varying degrees of increased glomerular permeability. This concept is in accord with most experimental observations but a certain amount of evidence has been advanced which suggests that the fault may not lie entirely in the glomerular filter, and that the plasma proteins may be primarily altered in such a manner as to render their elimination from the body not only possible but necessary. It is well recognized that, whereas normal plasma protein is retained in the blood, foreign protein entering the blood plasma is readily eliminated by the kidney. Thus hemoglobin, when free in the plasma, and egg albumin and other foreign proteins, if introduced into the blood stream, are promptly eliminated in the urine. In the case of hemoglobin, at least, some of the hemoglobin filtered through the glomeruli is reabsorbed in the tubules, appearing in the urine only after the reabsorptive capacity of the tubules has been exceeded (p. 178). Whether or not proteinuria produced in this manner is dependent upon renal injury has not been definitely established, although the observation that there is usually an associated excretion of native plasma protein would seem to indicate that such is the case.

It has been suggested that the plasma proteins in glomerulonephritis and nephrosis differ from those in normal individuals. Chemical and immunological data have been produced in support of this hypothesis. It has been reported that the albumin fraction of the serum of nephrotic patients is not homogeneous, as is normal serum albumin, but contains molecules of varying size. Since the subfractions of lower molecular size pass more abundantly into the urine, the mean molecular weight of the urine albumin may be only one-half that of the serum albumin and that of the urine globulin only one-third that of the serum globulins.

However, more recent studies, with improved analytical technics, have failed to reveal the presence of significant qualitative abnormalities in plasma or urinary proteins in patients with glomerulonephritis.

Fischer believed that urinary proteins are derived exclusively from the renal cells. Whereas it seems likely that protein may appear in the urine as a result of extensive degeneration of renal epithelium, it is quite evident that the quantity so derived must be extremely small as compared with that which comes from the blood plasma. Furthermore, in spite of the fact that the most marked degrees of proteinuria are found in those disorders in which the demonstrable pathologic lesions are predominantly those of degeneration of the tubular epithelium, the weight of experimental evidence supports the view that by far the greatest part of the urinary protein is derived by passage of plasma proteins through the glomerular membrane.

Since the urine normally contains minute quantities of albumin, the term "albuminuria" implies rather a quantitative than a qualitative deviation from the normal. This term is applied to the presence in the urine of abnormally large quantities of albumin, detectable by the commonly employed qualitative tests.

Functional Albuminuria. Under this heading are placed cases of albuminuria apparently not dependent upon organic disease. The urine is otherwise normal, renal functional activity is unimpaired, the degree of albuminuria is usually slight (below 500 mg. per 100 cc.), the condition is usually transitory and occurs most commonly in young individuals, whose subsequent medical history is negative.

(1) FOLLOWING SEVERE EXERCISE. Temporary albuminuria of varying degree may occur following strenuous muscular exercise in individuals unaccustomed to such activity. It has been observed in raw recruits after forced marches, in football players, bicycle racers, marathon runners and other athletes if the degree of exertion exceeds that to which the individual is accustomed. This type of functional albuminuria may be associated with the appearance of casts and even red blood cells in the urine. It is questionable whether the term "functional" is applicable to such cases, as the presence of temporary renal damage cannot be ruled out. However, these individuals are otherwise normal in every respect and the condition is transitory.

(2) SEVERE MENTAL STRAIN.

(3) PROLONGED EXPOSURE TO COLD (cold baths).

(4) ALIMENTARY ALBUMINURIA. The ingestion of excessive quantities of native protein, particularly egg albumin, may be followed, in two to three hours, by the appearance of albumin in the urine.

(5) ESSENTIAL ALBUMINURIA. Posner proposed the term "essential albuminuria" to include those cases variously designated as albuminuria of adolescence, cyclic albuminuria, postural or orthostatic albuminuria and intermittent albuminuria. This type of albuminuria is of great clinical importance because of its comparative frequency and because of the fact that it is commonly erroneously considered to be an evidence of renal disease. It is usually first discovered in the course of routine examination of applicants for insurance.

The condition occurs most frequently between the ages of fourteen and eighteen, more commonly in boys than in girls, particularly in those presenting manifestations of autonomic imbalance and vasomotor instability. It may be present in several members of the same family. The blood pressure, particularly the diastolic pressure, is frequently low. Lordosis is a prominent feature in certain cases. The term "orthostatic" is applied to those cases in which albuminuria is present during periods of ordinary activity in the erect posture and disappears upon the assumption of the recumbent posture. Heredity, lordosis, abnormal renal circulation, movable kidney, and vasomotor instability appear to be important etiologic factors in many of these cases. In certain instances the condition persists into adult life, in others it disappears spontaneously.

As in most of the previously mentioned types of "functional" albuminuria, the essential etiologic factor is probably some disturbance of renal circulation, either in the large vessels (movable kidney, lordosis) or in the renal arterioles (vasomotor instability). The occurrence of temporary albuminuria following experimentally produced circulatory changes in the renal vessels has been frequently demonstrated. The clinical importance of this type of albuminuria lies in the fact that it is not associated with nor followed by renal disease. So far as is known, these individuals are not predisposed to the development of nephritis or nephrosis, and the expectation of life is not affected by the presence of essential albuminuria.

(6) PREMENSTRUAL ALBUMINURIA.

(7) ALBUMINURIA OF PREGNANCY. Albuminuria may occur in 30 to 50 per cent of women during uncomplicated pregnancy and labor, disappearing immediately after parturition.

Organic Albuminuria. Albuminuria may result from (*a*) qualitative alteration in the plasma albumin (p. 171), usually but not necessarily in association with a glomerular lesion, (*b*) renal changes secondary to abnormalities in organs other than the urinary tract (prerenal albuminuria), (*c*) primary renal disease (renal albuminuria), and (*d*) disease of the lower urinary passages and contiguous structures (postrenal albuminuria).

The organic causes of albuminuria may be conveniently discussed under three headings: (*a*) Prerenal, (*b*) postrenal, and (*c*) renal.

✓ (*a*) PRERENAL ALBUMINURIA. The term "prerenal" cannot be applied in a strict sense, for most of the prerenal factors which cause albuminuria do so by producing changes in the kidneys. However, from the standpoint of the primary condition the use of this term is justifiable and appears in many respects advantageous.

(1) *Cardiac Disease.* In the decompensated stages of myocardial disease, with passive congestion of the kidneys, albuminuria of varying degree develops. The quantity of albumin eliminated is directly dependent upon the degree of renal circulatory embarrassment, and, unless the state of passive congestion is unduly prolonged, with consequent organic change in the kidneys, the albuminuria disappears upon reestablishment of circulatory efficiency.

(2) *Ascites* due to local intra-abdominal disease, unassociated with nephritis or

myocardial failure, may cause albuminuria, presumably by pressure upon the renal veins, causing renal congestion.

(3) *Intra-Abdominal Tumors* may produce albuminuria if they are so situated as to cause pressure upon the renal veins.

(4) *Febrile Albuminuria.* Fever, regardless of the cause, may be associated with slight albuminuria. This is due to the production of slight glomerular and tubular changes which are not permanent, being essentially nephrotic rather than nephritic in nature (cloudy swelling) and rapidly subsiding following the restoration of normal body temperature. Febrile albuminuria is commonly observed in pneumonia, typhoid fever, rheumatic fever, malaria, and the acute infectious diseases of childhood. In the latter, particularly in scarlet fever, this condition must be carefully differentiated from a complicating nephritis.

(5) *Convulsive Disorders.* Albumin may appear in the urine during and after convulsions from any cause, including brain tumor, tetanus, epilepsy, and meningitis. Albuminuria may also occur in coma due to cerebral vascular accidents, particularly cerebral or meningeal hemorrhage, in which conditions large quantities of albumin may be eliminated (1–2 gm. per 100 cc.)

(6) *Diseases of Blood.* Albuminuria of slight or moderate degree may occur in association with profound anemia, leukemia, and purpura hemorrhagica. It is perhaps due to nutritional changes in the kidneys (nephrosis).

(7) *Hyperthyroidism.* Albuminuria in hyperthyroidism is probably due to nephrosis or to vasomotor instability, as in the case of certain types of functional albuminuria.

(8) *Intestinal Obstruction.* Albuminuria is present in most cases of acute intestinal obstruction (see Intestinal Obstruction, p. 283), It may be dependent upon renal changes resulting from the presence in the blood stream of toxic protein products or from the state of alkalosis which is a prominent feature of the condition.

(9) *Hepatic Disease and Jaundice.* Jaundice is not infrequently accompanied by the elimination of small quantities of albumin in the urine. This has been attributed to a toxic or irritant effect of the circulating bile pigments or bile acids upon the renal glomerular membrane or the tubular epithelium. In addition to this factor, it may be that the existing state of hepatic dysfunction or functional insufficiency results in some alteration in the physicochemical state of the plasma proteins which causes them to be eliminated by the kidneys. This problem was referred to in discussing the nature and origin of urinary proteins.

(10) *Drugs* which cause renal irritation may give rise to albuminuria. Among these are turpentine, cantharides, phosphorus, arsenic, mercury, quinine, copaiba, salicylic acid, phenol, bismuth, lead, ether, chloroform, chromates, oxalates, zinc, opiates, apiol, sandalwood oil, lysol, naphthols, radium, squill, barbiturates, and sulfonamides. In the majority of instances the renal lesion is largely degenerative in nature (nephrosis).

(11) *Lipoid Nephrosis.* Because of the existing discordant views regarding the essential nature of this disorder, it will be considered in the group of albuminurias of renal origin.

(*b*) Postrenal Albuminuria. Under this designation may be placed the causes of so-called "false" albuminuria, a term frequently applied to the addition of albumin to the urine at some point beyond the uriniferous tubule. This group includes, therefore, inflammatory and degenerative lesions of the renal pelvis, ureter, bladder, prostate, and urethra. The possibility of contamination of the urine by vaginal secretions and discharges must be carefully excluded in considering the cause of albuminuria in women. The commonly employed tests for albumin will, of course, yield positive results in the presence of blood, the source of which frequently resides in the lower urinary tract. Inflammatory exudates are rich in protein and are therefore often responsible for albuminuria of this type. Consequently, urine containing pus will practically always contain albumin. It has been estimated that for each 100,000 leukocytes per 2 cc. of urine, 0.1 per cent of albumin may be expected. This observation is at times of importance in determining the presence or absence of albumin of renal origin in urine containing pus resulting from a lower urinary inflammatory process.

(*c*) Renal Albuminuria. (1) *Destructive Lesions of Kidney.* Albuminuria may occur in tuberculosis, carcinoma, pyelonephritis and polycystic disease of the kidney, and in hypernephroma and other lesions which are characterized by destruction or invasion of kidney tissue. In many cases, however, the urinary findings are normal. Renal infarction is usually associated with albuminuria which is at first marked but rapidly subsides.

(2) *Glomerulonephritis and Nephrosclerosis.* Albuminuria is an almost constant feature of acute glomerulonephritis. It may be of moderate or severe degree; the amount of albumin does not necessarily parallel the severity of the condition. Albuminuria is one of the most persistent manifestations of acute nephritis, being usually present after other manifestations (except microscopic hematuria) have disappeared. In the latent stages of glomerulonephritis, albuminuria may be the only indication of the renal lesion. In the early stages of chronic glomerulonephritis large quantities of albumin may be eliminated over long periods of time. With the development of impairment of renal function the albuminuria diminishes and in the later stages of the disease may be extremely slight. This change is due to the progressive impairment of the ability of the kidney to eliminate not only the substances normally present in the urine but also plasma albumin and other proteins. There is evidence that one of the factors concerned in the pathogenesis of renal insufficiency in many cases of glomerulonephritis is mechanical obstruction of glomeruli and tubules by accumulations of fibrin and hyaline protein coagula, consisting chiefly of precipitated globulins.

In nephrosclerosis (essential hypertension), albuminuria varies both in incidence and in degree. In many patients with essential hypertension, albuminuria is absent except during periods of myocardial failure with renal congestion. It may be intermittent and slight in amount. In so-called "malignant" hypertension, with rapid progression of the vascular changes in the kidney and with consequent nutritional changes in the glomeruli, relatively large quantities of albumin may be eliminated. With increasing renal functional impairment, however, albuminuria may diminish, as in the case of chronic glomerulonephritis.

(3) *Nephrosis* (see p. 620). The term "nephrosis" has been applied to diseases characterized anatomically by primarily degenerative lesions of the renal parenchyma. The nephroses may be subdivided into four groups: (*a*) larval nephroses, including those forms due to fevers, diabetes, jaundice, hyperthyroidism, pernicious anemia, and various drugs; (*b*) necrotizing nephroses, including those forms due to chemical agents, chiefly mercury, bismuth, and, less frequently, arsenic and the barbituric acid series of hypnotics, and those due, in rare instances, to severe acute infections (cholera, typhoid fever, diphtheria); (*c*) chronic nephrosis, the most characteristic form being the nephrotic stage of chronic glomerulonephritis; (*d*) amyloid disease of the kidneys.

Albuminuria is a constant feature of nephrosis, except in those acute necrotizing forms which are frequently accompanied by anuria. If any urine is eliminated, it will practically always be found to contain albumin. The quantity of albumin varies. In the mild forms of larval nephrosis and in certain cases of necrotizing nephrosis albuminuria may be slight; in other cases, however, it may be marked. Large amounts of albumin are usually excreted in lipoid nephrosis and in amyloid disease of the kidneys. Only in rare instances are small quantities eliminated. As has been mentioned previously, the urinary protein in these conditions consists largely of albumin, the albumin : globulin ratio being high (above 10). If an existing nephrosis, with purely or predominantly tubular lesions, becomes complicated by a superimposed nephritis with glomerular lesions, the albuminuria may diminish, and, with progressive renal functional impairment, only small quantities of albumin may be eliminated, the urinary picture being similar to that observed in chronic glomerulonephritis.

(4) *Eclampsia.* Large amounts of albumin may be eliminated in true eclampsia, unassociated with glomerulonephritis or nephrosis. This is probably dependent upon renal vascular damage or, perhaps, may result from the hepatic lesions which constitute an important pathologic feature of that condition.

Quantity of Protein in Urine. As has been indicated, the amount of protein eliminated in the urine is exceedingly variable. Addis believed that the upper limit of normal proteinuria is about 30 mg. for the twelve-hour-day period. Others state that as much as 75 mg. may be eliminated in twenty-four hours by normal individuals under normal conditions. In benign, functional or physiologic albuminuria, the mixed twenty-four-hour specimen seldom contains more than 0.2 per cent of protein, although in some cases as much as 0.5 per cent has been observed. Immediately after severe exercise, however, and in orthostatic albuminuria shortly after assuming the erect posture, specimens of urine may contain 1 per cent or more of protein.

The largest amounts of protein are usually present in the urine of patients with chronic nephrosis and amyloid nephrosis, in which conditions 2 to 5 per cent or more of protein may be eliminated. More than 110 gm. have been eliminated in twenty-four hours, the usual quantity varying from 5 to 60 gm. with an average daily loss of 15 to 20 gm. Some investigators have observed an increase in urinary protein in these cases during periods of excessive protein intake. This is particularly true in those instances in which the daily output is greater than 20 gm.

In most cases of nephrosis, however, large amounts of protein may be ingested without an associated increase in albuminuria; in certain instances of lipoid nephrosis, albuminuria may diminish following the administration of a high protein diet with consequent increase in the concentration of albumin in the blood plasma.

In acute glomerulonephritis the urinary protein usually varies from 0.2 to 1.0 per cent, but may exceed 2 per cent in rare instances. In chronic glomerulonephritis and in essential hypertension the figures usually vary from 0.2 to 0.5 per cent; in some cases, particularly in those instances of nephritis with a nephrotic component, the urinary protein may be greatly increased and approach the amounts observed in pure nephrosis. With the supervention of renal insufficiency, however, the quantity of protein in the urine in all types of nephritis is usually diminished. Increases in proteinuria in Bright's disease may be explained by the presence of one or more of the following factors: (*a*) increase in glomerular permeability; (*b*) increase in the rate of glomerular filtration; (*c*) the presence in the diet or the body reserves of more new material from which plasma proteins may be constructed; (*d*) artificial increase in the concentration of plasma protein, such as follows transfusion. It would appear that, in addition to increased severity of damage to the glomerular capillaries, an increase in the amount of protein in the urine may be effected in some instances by an increase in protein intake and even by actual improvement in renal function, as indicated by the urea clearance test. Such findings emphasize the fact stated above, that the quantity of albumin in the urine does not parallel the severity of renal damage.

In cases in which the urine contains large quantities of protein, this substance may have a significant effect upon the urinary specific gravity. One gram of albumin per 100 cc. increases the specific gravity by 0.003. Under such circumstances, correction must be made for the amount of albumin in the urine when performing urine concentration tests, which depend upon accurate estimation of urinary specific gravity. If such correction is not made, results will be obtained which may be misleading.

Other Proteins in Urine. NUCLEOPROTEIN IN URINE. Nucleoprotein is present in the urine in abnormal amounts in inflammatory conditions of the lower urinary passages, particularly cystitis and pyelitis; it may also be found in nephritis. The term "nucleoprotein" is incorrectly applied to include other protein substances such as mucin and phosphoprotein, which may appear in the urine in increased amounts.

PROTEOSES AND PEPTONES IN URINE. Proteoses and peptones (i.e., polypeptides) may be found in the urine in certain febrile disorders, particularly pneumonia, diphtheria, and pulmonary tuberculosis, as well as in peptic ulcer, carcinoma, and osteomalacia.

BENCE JONES PROTEINURIA. What is termed Bence Jones protein is now regarded as a group of proteins, apparently globulin in nature, of comparatively low molecular weight (about 37,000), that possess the peculiar properties of precipitating from solution at 40 to 60° C. and of redissolving, partially or completely, at 95 to 100° C. They occur in the urine in multiple myeloma, in which condition they are frequently of considerable diagnostic significance. They have also been reported in the urine of patients with extensive osteosarcoma, carcinomatous metastases to bone, hyperparathyroidism, comminuted fractures, senile osteomalacia, lymphocytic and myelocytic leukemia, and polycythemia. Recent studies suggest that Bence Jones proteinuria occurs in conditions other than multiple myeloma probably only when there is extensive bone marrow involvement or reaction to the disease process.

As indicated above, this protein is of significance mainly in multiple myeloma. There is evidence that it is probably produced by the myeloma cells. The incidence of Bence Jones proteinuria in multiple myeloma has been reported by various authors to be from 8 to 87 per cent. It was observed in 53 per cent of cases in a recent large series. One reason for this variability is probably the fact that it may occur intermittently in early stages of the disease, although when it appears later it usually persists. It is accompanied by albuminuria in about 80 per cent of cases, but the latter may occur without Bence Jones proteinuria (30–40% of cases).

Although these proteins are presumably brought to the kidneys in the blood plasma, there being no evidence that they are necessarily formed in the kidneys, they have seldom been demonstrated in the blood stream, although a variety of abnormal globulin patterns occur commonly in multiple myeloma (p. 161). It has been suggested that their small molecular size may permit their passage through the glomeruli at plasma concentrations too low to permit detection in that medium by available methods.

There is no consistent correlation between the occurrence of Bence Jones proteinuria and abnormalities in the plasma globulins in this disease, as indicated by the following observations: Bence Jones proteinuria has been found to occur in only 50 to 60 per cent of cases with hyperglobulinemia and, conversely, 15 to 30 per cent of patients with Bence Jones proteinuria exhibit minor or no significant plasma protein abnormalities.

HEMOGLOBINURIA. (p. 207). Hemoglobinuria is the term applied to the presence of free hemoglobin in the urine, in contradistinction to hematuria which refers to the presence of red blood corpuscles in the urine. The excretion of hemoglobin by the kidneys is almost invariably preceded by the occurrence of hemoglobinemia, an excessive amount of free hemoglobin in the circulating blood plasma. Hemoglobinuria is therefore dependent upon excessive hemolysis. It has been estimated that it occurs only after the plasma contains more than 60 to 150 mg. of free hemoglobin per kilogram of body weight. Up to this point, the hemoglobin that is filtered through the glomeruli is completely reabsorbed in the tubules; above these levels, the reabsorptive capacity of the tubular epithelium for hemoglobin is exceeded and the excess appears in the urine. The clinical conditions in which this occurs are presented elsewhere (Hemoglobinemia, p. 207).

Before making a diagnosis of hemoglobinuria it is, of course, essential to exclude the possibility that the condition may in reality be hematuria, the red corpuscles having been partly or completely hemolyzed in the bladder or after elimination of the urine. This is particularly apt to occur in alkaline or ammoniacal urine.

Urinary Nonprotein Nitrogen

Investigation of the nonprotein nitrogenous constituents of the urine in disease has been largely superseded by the quantitative determination of these substances in the blood. However, particularly in nephritis, the study of both blood and urinary nonprotein nitrogenous elements may yield valuable information. Since the amount of urinary nitrogen depends primarily upon the quantity of protein ingested, it is obvious that no clinical significance can be attached to the nitrogen output unless the intake be determined simultaneously. Even under such circumstances accurate information cannot be obtained in many instances because of the fact that nitrogen equilibrium may be established at various levels of blood nonprotein nitrogen. Before drawing conclusions from alterations in urinary nitrogen, therefore, several factors must be carefully investigated.

Urinary Urea. Urea administered to normal individuals is promptly eliminated because the storage capacity of the body for nitrogen is limited. In certain patients with nephritis the elimination of excessive amounts of ingested urea may be delayed for varying periods of time. In some cases, however, no deviation from the normal can be demonstrated. In patients with chronic nephritis in whom retention of nitrogen occurs following the ingestion of excessive quantities of protein, the retained nitrogen frequently cannot be entirely accounted for by the concentration of nonprotein nitrogen in the blood, which may or may not be increased; it is believed that in such cases the excess nitrogen is retained in the tissues, chiefly in the liver and muscles.

In many cases of nephritis the ability of the kidneys to concentrate urea is impaired although the amount eliminated in twenty-four hours may be normal. The normal individual is able to concentrate urea to the extent of 2 per cent or more in the second hour after the ingestion of 15 gm. of urea dissolved in 100 cc. of water. If renal function is impaired the urea concentration in the urine is below this figure (see p. 598).

The most important clinical applications of studies of urea elimination have resulted from studies of urea clearance. It has been shown that with urine volumes below 2 cc. per minute (the augmentation limit) the blood urea clearance increases in direct proportion to the square root of the urine volume. With urinary outputs below this figure the volume of blood which is cleared of urea in one minute was found to vary, in normal individuals, from 41 to 65 cc., with a mean of 54 cc. (standard clearance). When the rate of urine excretion exceeds 2 cc. per minute, the urea clearance, having attained its maximum at that point, is unaffected by further increase in urine volume. Under such circumstances, the volume of blood which is cleared of urea in one minute was found to vary from 64 to 99 cc. with a mean of about 75 cc. (maximum clearance) (see p. 599). Decrease in urea clearance may be demonstrable in patients with renal disease before evidence of renal functional impairment can be obtained by any other method. This subject will be considered in greater detail in the discussion of tests of renal function (p. 602).

In hepatic insufficiency, owing to decreased formation of urea from amino acids, the proportion of urinary nitrogen occurring as urea may be decreased. Urea normally constitutes about 60 per cent of the total urinary nitrogen on a low protein diet and 90 per cent on a high protein diet, the average figure being about 80 per cent. Figures of 50 per cent may be obtained in acute hepatic disease even with an adequate protein intake. In many cases, however, the urinary nitrogen partition is normal.

A relative decrease in the urinary urea fraction may also occur in conditions associated with severe acidosis. Under such circumstances the low proportion of urea is due to the great increase in urinary ammonia, formed by the kidney, which is an important feature of the mechanism of regulation of acid-base balance (p. 331). The urine urea N decreases (positive N balance) following administration of growth hormone, testosterone, or insulin; there is a simultaneous fall in the concentrations of urea and amino acids in the plasma. This is a reflection of stimulation of protein synthesis by these agents. Administration of 11-oxygenated adrenocortical hormones or excessive doses of thyroxine increases urea excretion (negative N balance), a reflection of increased protein catabolism.

An increase (above nitrogen intake) in the amount of urea eliminated in the urine may occur in disorders associated with excessive tissue catabolism, as in prolonged wasting diseases and febrile states, urea (or urea plus ammonia) comprising by far the largest portion of the increased nitrogen output. The outstanding exception is severe hepatic necrosis, in which the deamination mechanism in the liver is impaired, resulting in excretion of relatively large amounts of amino acids and correspondingly smaller amounts of urea (as low as 10–50 per cent of the total urine N).

Uric Acid in Urine. The factors which influence excretion of uric acid are considered in the discussion of nucleic acid metabolism (p. 199).

Creatine and Creatinine in Urine. The excretion of creatine in the urine under normal conditions has been discussed elsewhere (p. 150). The investigation of this factor is of significance particularly in the study of metabolic changes in certain myopathies because of the important relation of creatine metabolism to muscular function. Early observations established the presence of an unstable compound containing phosphoric acid and creatine in fresh, excised muscle, which soon began to disappear and, in a few hours after removal of the muscle, was no longer demonstrable; as it disappeared, free phosphoric acid and creatine appeared in molecular ratio to one another. This unstable compound of phosphoric acid and creatine was diminished by stimulation. Stimulation during arrest of the circulation through the muscle caused the compound to break up completely and, when the blood supply was restored and the muscle allowed to rest, the combination of phosphoric acid with creatinine was once more renewed, the quantity of the compound increasing steadily during the period of rest. Observations such as these indicate that creatine has an important bearing on the mechanism of muscular contraction (p. 11).

Excessive creatinuria may occur in starvation, febrile and wasting diseases, diabetes mellitus, in eunuchoids and castrates, in postencephalitic and Parkinsonian rigidity, and rather consistently, but not invariably, in certain myopathies (myotonia congenita, myasthenia gravis, various forms of myositis, congenital muscle dystrophies, congenital muscular hypertrophy, and secondary muscle atrophy). Testosterone diminishes creatinuria in eunuchoids and castrates, apparently by increasing the storage, but not the synthesis, of creatine. On the other hand, administration of methyltestosterone, after a latent period of four to sixteen days, is followed by increased excretion of creatine and creatinine, due probably to increased synthesis of creatine. Increased creatine excretion follows intravenous administration of amino acid digests.

Of particular interest in this connection is the influence of glycine upon the clinical and metabolic course of certain of these myopathies, particularly myasthenia gravis. The administration of large doses of glycine results, in certain of these cases, in a 100 to 1000 per cent increase in creatine excretion. A similar increase in creatinuria also occurs in the majority of normal subjects after the ingestion of glycine. After a period of several weeks the creatinuria begins to decrease in most instances, eventually falling to almost control levels despite the continued administration of glycine. The observation has been made that cases of primary myopathy in which the average creatinuria increased more than 50 per cent above the control level after the administration of glycine showed both subjective and objective improvement, provided this increased creatinuria disappeared within a few weeks. Little or no improvement was observed in cases in which the increase in creatinuria was less than 50 per cent.

Excessive creatinuria has also been observed in hyperthyroidism, following the administration of thyroid extract, during the menstrual period and in pregnancy, and when carbohydrate is excluded from the diet. It has also been observed after fractures (to 500 mg. daily). It disappears gradually during the period of healing of the fracture and after the resumption of activity.

As has been stated (p. 150), the output of creatinine is remarkably constant under normal conditions and is essentially independent of diet and muscular activity. The elimination of creatinine is increased in conditions associated with increased tissue catabolism, as in fevers, and is decreased in the presence of marked muscular atrophy. It has been found that in the primary myopathies the diminished creatinuria which occurs as a secondary phenomenon following the administration of glycine is accompanied by an increased excretion of creatinine and an improvement in the patient's ability to retain ingested creatine.

Preformed creatinine, administered to normal individuals, is promptly eliminated.

Creatine Tolerance. When 1.32 or 2.64 gm. of creatine hydrate (equivalent to 1 and 2 gm., respectively, of creatine) are ingested by a normal adult, about 80 per cent is retained by normal men and about 70 per cent by normal, nonpregnant women; about 20 and 30 per cent, respectively, are excreted in the urine during the subsequent twenty-four hours. Diminished tolerance (i.e., excessive excretion) is present in conditions accompanied by spontaneous creatinuria, mentioned above, and in mild cases of these disorders in the absence of creatinuria under ordinary dietary conditions.

Creatine tolerance is decreased in the majority of patients with hyperthyroidism and tends to be increased (excessive retention of administered creatine) in hypothyroidism. This procedure may be of value in the diagnosis of hyperthyroidism or hypothyroidism in children, in whom it may be difficult to secure accurate determinations of basal metabolic rate. In children, correction must be made for normal creatinuria, which averages 4.2 mg. daily per kilogram of body weight on a meat-free diet, providing adequate calories and 2 gm. of protein per kilogram.

Amino Acids in Urine. Excessive aminoaciduria may occur in conditions in which the plasma amino acid concentration is increased or in which there is defective reabsorption of these substances in the renal tubules. These abnormalities may apply to all amino acids or be confined to certain groups or to single amino acids or their metabolites.

WASTING DISEASES. Urine amino acids may be increased in conditions in which there is extensive tissue wasting, as in protracted fevers (typhoid, tuberculosis) and malignancy, or in which there are areas of extensive tissue autolysis.

HEPATIC DISEASE. Increase in urinary excretion of amino acids occurs, but not consistently, in acute and subacute hepatic necrosis, e.g., "acute yellow atrophy," eclampsia, and in poisoning with carbon tetrachloride, chloroform, cinchophen, arsenic, and phosphorus. The increase includes a wide variety of amino acids, some of which, because of limited solubility, may crystallize out of solution (viz., leucine and tyrosine crystals). This increased elimination is due in part to decreased deamination of amino acids in the damaged liver, and in part to extensive autolysis of liver cell proteins, with consequent excessive production of amino acids. The blood amino acid concentration is increased in these cases.

Similar findings have been obtained in 20 to 30 per cent of patients with cirrhosis of the liver, the quantity of urinary amino acids roughly paralleling the activity of the disease in these cases. This phenomenon has been attributed to lowered rates of deamination, conjugation, or utilization of these substances. The increase apparently usually involves mainly methionine, valine, tyrosine, and tryptophan; less frequently, leucine, threonine, arginine, and phenylalanine. Values for lysine and histidine are often low. Although clinical improvement may be reflected at times in decrease in amino acid excretion, in general the degree of aminoaciduria has little if any prognostic or diagnostic significance.

HEPATOLENTICULAR DEGENERATION (WILSON'S DISEASE). Recent studies suggest that aminoaciduria occurs rather consistently in this disease. This phenomenon, which involves apparently most if not all of the amino acids found normally in the urine, is not necessarily associated with a significant increase in plasma amino acids, nor is it related to the extent of liver damage or of hepatic functional impairment as demonstrable by other methods. It is due apparently to defective reabsorption of amino acids by the renal tubular epithelial cells, as in the Fanconi syndrome, and, like the latter condition, may be accompanied in certain cases by glycosuria and osteomalacia, i.e., defective renal reabsorption of glucose and phosphorus.

FANCONI SYNDROME. This designation is applied to a rather characteristic group of biochemical abnormalities, apparently due fundamentally to defects in certain renal tubular reabsorptive mechanisms. In its most complete form, this syndrome is characterized by: (*a*) aminoaciduria; (*b*) ketonuria; (*c*) increased urinary excretion of other organic acids, e.g., lactic; (*d*) acidosis (p. 244); (*e*) glycosuria (p. 62); (*f*) increased urine phosphate with hypophosphatemia and rickets or osteomalacia (p. 244); (*g*) increased urinary ammonia (i.e., acidosis). Not all of these abnormalities necessarily occur in every case.

Amino acids comprise the largest fraction of the increment in urinary organic acids, and an abnormally high percentage of the total urinary nonprotein nitrogen (up to 15 per cent; normal usually below 3 per cent). Most of the usual amino acids are apparently involved in this increase, which, together with that in other organic acids, is largely responsible for the production of acidosis in this condition, i.e., by causing excessive loss of cations (Na, K, Ca, Mg) in the urine.

In the conditions outlined above, the excessive aminoaciduria appears to involve most if not all of the amino acids found normally in the urine. Under certain circumstances, there is increased excretion of specific amino acids or certain of their metabolites.

TYROSINURIA AND PHENYLKETONURIA. The normal metabolic degradation of tyrosine and phenylalanine involves the formation of p-hydroxyphenylpyruvic, p-hydroxyphenyllactic, dihydroxyphenylpyruvic, p-hydroxyphenylacetic, and dihydroxyphenylacetic (homogentisic) acids. The latter subsequently forms acetoacetic acid and a Krebs cycle intermediate (fumaric acid). Defects may occur at various points in this metabolic sequence, due, in some instances at least, to inherent enzyme deficiencies ("inborn errors of metabolism"), with the consequent urinary excretion of increased amounts of certain intermediary metabolites of tyrosine and phenylalanine.

(1) *Alkaptonuria.* This condition is characterized by the urinary excretion of homogentisic acid (dihydroxyphenylacetic acid), due apparently to lack of an enzyme essential for the next step (oxidation) in the metabolism of this substance. This anomaly is often discovered in infancy, since the homogentisic acid in the urine, on exposure to air, is readily oxidized to dark-colored compounds, especially if the urine, on standing, becomes alkaline due to ammoniacal fermentation. It is probably present at birth and persists throughout life, apparently with no directly harmful consequences, despite the fact that several grams of homogentisic acid may be excreted daily. In later life, accumulation of dark pigment in cartilages and tendons may give rise to the condition known as ochronosis, which may be accompanied by arthritic changes.

(2) *Tyrosinosis.* This scarcely merits independent consideration, inasmuch as only one case has been reported. It is characterized by the urinary excretion, on an ordinary diet, of p-hydroxyphenylpyruvic acid, with excretion of tyrosine and

p-hydroxyphenyllactic acid after ingestion of large amounts of tyrosine. The biochemical defect obviously occurs earlier in the metabolic sequence than in the case of alkaptonuria.

(3) *Phenylketonuria.* This condition, also known as phenylpyruvic oligophrenia, imbecillitas phenylpyruvica, and phenylpyruvia, is an "inborn error of metabolism" occurring in certain mentally defective individuals, characterized by the urinary excretion of phenylalanine and its direct metabolites, phenylpyruvic, phenyllactic, and phenylacetic acids. Phenylpyruvic usually predominates, which, since pyruvic is a ketone acid, is the reason for the designation, phenylketonuria, a term not applicable to the other acids. The underlying defect is apparently in some component of the enzyme system involved in the conversion of phenylalanine to tyrosine (oxidation), the major normal metabolic pathway. This defect results in the presence of excessive amounts of phenylalanine in the blood stream. In the kidneys, the excess undergoes deamination, and reduction or decarboxylation, to produce the substances mentioned above, which are excreted in the urine, together with phenylalanine. The significance of this metabolic abnormality in relation to the mental deficiency is not known.

Phenylpyruvic and phenyllactic acids, together with the corresponding p-hydroxyacids (see alkaptonuria), have been found in the urine of premature infants given diets rich in protein (i.e., tyrosine and phenylalanine) and deficient in ascorbic acid. This may occur also at times in adults and in patients with pernicious anemia under similar dietary conditions. Administration of adequate amounts of ascorbic acid corrects this condition, but has no effect on the excretion of these acids in either alkaptonuria or phenylpyruvic oligophrenia.

Cystinuria. This has been generally regarded as an "inborn error of metabolism," in which cystine is present consistently in the urine in abnormally large amounts (0.4–1 gm. daily; normal, less than 0.1 gm.). It is a congenital disorder, with a strong familial tendency. Its chief clinical importance arises out of the relative insolubility of cystine at usual urine pH levels, which may result in the formation of cystine calculi. These may grow to enormous size in the bladder. Explanation of the mechanism of cystinuria is a controversial matter. Administration of methionine or cysteine to a cystinuric results in increased excretion of cystine, whereas administration of cystine itself does not. Until recently, these curious findings were explained on the highly unsatisfactory basis that the interconversion of cystine and cysteine is interfered with in cystinuria, and that cystine has a metabolic pathway independent of cysteine. A more satisfactory explanation is afforded by the observation that cystinurics excrete, in addition to cystine, abnormally large amounts of other diamino acids, viz., lysine, arginine, and ornithine. It has been suggested that the cystinuric inherits a defect in an enzyme system in the renal tubular cells involved in the reabsorption of compounds of this structural type from the glomerular filtrate. Cystine itself, when fed, is absorbed relatively slowly from the intestine and does not result in high levels of cystine in the blood. Methionine and cysteine, on the other hand, are rapidly absorbed and converted to cystine, presumably at a rate sufficient to raise its level in the plasma and glomerular filtrate beyond the point of complete reabsorption by the defective renal tubular mechanism. According to this view, cystinuria is a condition that comes under the category of a partial Fanconi syndrome (p. 183).

Cystinosis. This condition is apparently a metabolic disturbance which results in extensive deposition of the sulfur-containing amino acid, cystine, in various tissues, including the spleen, lymph nodes, liver, thyroid, intestinal mucosa, lungs, adrenals, pancreas, bone marrow, and kidneys. There is an associated acidosis; death occurs, usually as a result of renal insufficiency, at an early age. Although cystinuria may occur occasionally in this disorder, it usually does not, and the relationship, if any, between these two conditions remains obscure. They both exhibit a strong familial tendency. However, whereas cystinuria is a benign affair, except for the liability to develop urinary calculi, cystinosis is an extremely serious malady, probably invariably fatal.

There is some evidence to suggest that acidosis and hypophosphatemia, which may occur very early in cystinosis, before the supervention of renal failure, may be due to a defect in renal tubular reabsorption similar to that operating in the Fanconi syndrome. Some reported patients had renal glycosuria, aminoaciduria, and rickets, which would place them in this category (p. 183).

Melanuria. Melanin is a dark, brown-black, sulfur-containing pigment, normally found in the hair, skin, ciliary body, choroid of the eye, pigment layer of the retina, and various nerve cells. Its exact chemical structure is not known, but it apparently is derived from tyrosine which, through the action of tyrosinase, forms dihydroxyphenylalanine (dopa), the latter, a colorless substance, giving rise to a series of oxidation products terminating in melanin.

Melanuria occurs almost exclusively in the presence of melanotic sarcoma (in about 20 per cent of cases), usually when extensive liver metastases are present. It may be regarded as presumptive evidence of this condition although it has been reported, in rare instances, in terminal stages of hepatic cirrhosis and in intestinal obstruction, pernicious anemia, pneumonia, acute pancreatitis, and diabetes mellitus. It is occasionally accompanied by ochronosis.

A syndrome has been described, with a strong familial tendency, characterized by melanotic pigmentation of the oral mucosa, lips, and digits, associated with multiple intestinal polyposis, which frequently causes attacks of abdominal pain, intestinal obstruction, and intussusception. Melanuria has not been reported in patients presenting this syndrome.

Ammonia in Urine. The ammonia present in the urine is formed in the kidneys, from amino acids and glutamine, for purposes of neutralization of excreted acid (p. 331).

Urinary ammonia is increased in many conditions associated with acidosis (diabetes, starvation, dehydration, vomiting, diarrhea, etc.), this increase constituting one of the important compensatory mechanisms of the body whereby acids may be neutralized without the loss of excessive amounts of base from the body fluids (p. 331). The acidosis of nephritis, in which the ammonia-forming function of the renal tubular epithelium is impaired, constitutes one exception to this general rule. The ammonia content of the urine is usually decreased in alkalosis (p. 331). An increase may result from bacterial decomposition of urea in subjects with bladder retention and urinary tract infection (particularly cystitis).

Because of the role of urinary ammonia in the regulation of the acid-base equilibrium, the factor, ammonia plus titratable acid of the urine, may be utilized as an index of the state of this equilibrium, except in the presence of renal functional impairment or acidosis due to retention of carbonic or phosphoric acids. Normal and abnormal values, in terms of twenty-four-hour excretion of N/10 HCl + NH_3 per kilogram of body weight are as follows: normal resting adult, 0 to 27 cc.; mild acidosis, 27 to 65 cc.; moderate to severe acidosis, 65 to 100 cc.; severe acidosis, over 100 cc. (p. 336).

Negative Nitrogen Balance in Disease. Excessive protein catabolism, with consequent negative nitrogen balance, occurs in a great variety of abnormal states. Appreciation of the increased protein requirement of patients with these conditions has greatly improved their clinical management. It must be recognized that in the majority of instances the deficiency is not in protein alone. Various vitamins, electrolytes and fluid are also involved, and must be supplied in proper amounts if therapy is to be maximally effective. These therapeutic considerations are, however, beyond the scope of this volume.

Acute Infections. Most of the serious acute infectious diseases are accompanied by negative nitrogen balance, usually proportional to the severity of the disease, although not always to the height of the fever. This is not necessarily true of prolonged chronic infections, e.g., tuberculosis, in which N equilibrium can be maintained usually on a much lower caloric and protein intake than in acute infections with comparable temperature rises. It is perhaps significant in this connection that the BMR does not increase in these conditions in proportion to the rise in temperature (p. 375), as it does in acute infections.

Immobilization. A negative N balance occurs during periods of immobilization in bed. In healthy individuals, the urinary N begins to increase on about the fifth day, rising to a maximum in about ten days.

Burns. Negative N balance in patients with extensive burns is due in part, but not entirely, to loss of comparatively large amounts of protein in the exudate from the burned areas.

Operations, Fractures, Other Trauma. Major operative procedures in well-nourished subjects are followed promptly by negative N balance, accompanied by a drop in the concentration of plasma albumin, amino acids, and lipids. This does not occur in malnourished individuals and is much more consistent and more pronounced after intra-abdominal and serious intrathoracic operations than following more superficial procedures, e.g., herniorrhaphy.

Increased urinary N excretion occurs within a few days after traumatic fractures, and to a lesser extent after other types of trauma, normally roughly paralleling the severity of the injury. This is not entirely attributable to immobilization.

There is evidence which suggests that the disturbances in protein metabolism in the conditions outlined above are due in part to some metabolic aberration other than those resulting directly from disuse atrophy, tissue destruction, or heightened metabolism incident to fever. Hormonal mechanisms may be involved. Participation of the "alarm reaction" (p. 427) is suggested by the observation of the common occurrence of marked eosinopenia promptly after surgical operations and trauma, accompanied by an increase in urinary adrenal corticoids (p. 428) and, less consistently, 17-ketosteroids (p. 429). Moreover, negative N balance is not produced readily under these conditions in adrenalectomized animals. It appears, however, that factors other than the adrenal hormones are involved in the production of this phenomenon.

It must be borne in mind that although hypoalbuminemia is a clinically valuable reflection of a state of protein deficiency, its presence may be masked by coexisting dehydration (p. 152). Moreover, the extent of the total body protein deficit is not reflected quantitatively in the decrease in plasma albumin (p. 153). It has been shown that, in simple malnutrition, about 30 grams of tissue protein are lost for every gram lost from the plasma, indicating the high priority held by the plasma proteins in the general nitrogen pool.

Patients with protein malnutrition exhibit a number of undesirable tendencies, some of which are due to decrease in concentration of certain of the plasma proteins, others to impaired tissue protein synthesis. These include: (*a*) anemia; (*b*) edema; (*c*) increased susceptibility to hemorrhagic shock; (*d*) increased susceptibility to infection; (*e*) delayed wound healing; (*f*) delayed callus formation in fractures; (*g*) poor takes of skin grafts; (*h*) tendency to decubitus ulcers; (*i*) anorexia; (*j*) gastrointestinal motility disturbances; (*k*) increased susceptibility to liver damage.

Urinary Nitrogen Partition. The following table illustrates the relative amounts and proportions of the several nonprotein nitrogenous constituents of the urine excreted by normal individuals upon high and low protein diets.

Table 5. Influence of High and Low Protein Diets on the Relative Amounts of the Nitrogenous Constituents of the Urine

FOOD		COMPOSITION OF THE URINE IN GRAMS					
In grams	In calories	Urea *N*	Ammonia *N*	Uric acid *N*	Creatinine *N*	Undetermined *N*	Total *N*
Protein, 118 = 19*N* Fat, 148 Carbohydrate, 225	2786	14.70 57.5 per cent	0.49 3.0 per cent	0.18 1.1 per cent	0.58 3.6 per cent	0.85 4.9 per cent	16.8
Protein, 6 = 1*N* Fat, 52 Carbohydrate, 400	2153	2.20 61.7 per cent	0.42 11.3 per cent	0.09 2.5 per cent	0.60 17.2 per cent	0.27 7.3 per cent	3.6

Lusk, G.: Science of Nutrition, 4th ed., p. 251, W. B. Saunders Co., 1928. After Folin, O.: Am. J. Physiol., *13*:117, 1905.

This table reveals the fact that if a man ingests a diet containing a medium amount of protein, and again one that is nearly free from protein, the difference in the character of the urine in the two cases is almost exclusively due to a difference in the output of urea. The quantity of creatinine eliminated remains independent of the quantity of protein metabolized, and the same thing holds true, as a rule, for uric acid. Determination of the urinary nitrogen partition was at one time employed clinically particularly in the study of hepatic function. If hepatic function is impaired the proportion of urea N in the urine will be relatively low and that of amino acid N will be increased. In acidosis (except nephritic), urea N will be subnormal and ammonia and amino acid N increased. The study of the urinary nitrogen partition has been largely replaced by improved methods of study of renal and hepatic function and by more accurate methods of investigating alterations in the acid-base equilibrium.

Congo Red Test for Amyloidosis

Congo red, injected intravenously, is removed from the blood relatively slowly, about 70 to 90 per cent of the quantity injected remaining in the blood at the end of one hour in normal individuals. In the presence of amyloid disease the dye disappears from the blood more rapidly than normally, less than 40 per cent of the quantity injected remaining in the blood stream at the end of one hour. Under normal conditions practically all of the dye is excreted by the liver in the bile. The increased rapidity of its disappearance in amyloid disease is generally attributed to adsorption of the dye by the amyloid material and to its increased filtration from the blood through the damaged capillary endothelium.

This procedure is widely employed for the diagnosis of amyloid disease and is an extremely valuable test if certain complicating factors are taken into consideration. It has been found that the persistence of Congo red in the blood stream is dependent to a certain extent upon a normal concentration of plasma protein, particularly albumin, to which the dye is adsorbed. In the nephrotic syndrome, with marked albuminuria and a lowered plasma albumin concentration, the dye disappears from the blood with abnormal rapidity, as in the case of amyloid disease, a large proportion of it appearing in the urine, however, adsorbed to albumin. In the presence of marked albuminuria, therefore, the interpretation of subnormal retention of Congo red in the blood is difficult, but in amyloid disease much less of the dye appears in the urine as a rule than in the case of nephrotic syndrome. Occasionally, findings suggestive of the presence of amyloid disease are obtained in patients with low plasma albumin concentration in the absence of albuminuria. The Congo red test may be interpreted as strong confirmatory evidence of the presence of amyloid disease when more than 90 per cent of the dye disappears from the blood within one hour, in the absence of the elimination of significant amounts in the urine. In exceptional cases of amyloid disease this test may yield essentially normal findings, and findings suggestive of amyloid disease may be obtained occasionally in cases in which no amyloid substance is present. There is apparently no consistent relationship between the rapidity of removal of the dye from the blood and the extent of amyloid deposition in the tissues. The Congo red test is usually negative in primary amyloidosis, a rather rare condition.

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CHAPTER IV

Nucleic Acid Metabolism

The importance of the nucleic acids and their derivatives is of quite a different sort from that ascribed to the other classes of metabolites. It is doubtful whether significant quantities of energy are derived from the catabolism of nucleic acids; neither they nor their constituents are dietary essentials, and their only "structural" role appears to be in the composition of the chromosome. Rather, the chief role of nucleic acids and related compounds appears to be that of metabolic specialists, involved (1) in the control of the general pattern of metabolism and (2) in aiding the catalysis of certain particular reactions. The former category includes the genes, bacterial "transforming factors," viruses, embryological "evocators," and probably the cytoplasmic regulators of protein synthesis; in the latter are the adenylic system and the free nucleotides which act as coenzymes.

From the standpoint of protein chemistry, the nucleoproteins are a class of conjugated proteins, the prosthetic groups of which are the nucleic acids. As is the case with many other types of conjugated proteins (lipoproteins, glycoproteins, etc.), the chemical and biological properties of the prosthetic groups are at the present time the major objects of attention. While this justifies a consideration of these substances apart from the proteins proper, it may only reflect the current trend and be subject to future revision and reclassification.

Nucleic acids are substances of high molecular weight, containing phosphoric acid, sugars, and purine and pyrimidine bases. Two major chemical types of nucleoproteins have been found in nature, differing from each other in many respects. However, the difference which underlies the current system of nomenclature concerns the nature of the sugar contained in the nucleic acid moiety of the nucleoprotein. In one type of nucleoprotein the sugar is a pentose, which has been shown to be D-ribose in all cases which have been investigated to the point of isolation and final identification. The nucleic acid containing this sugar is called "pentosenucleic acid" (PNA) or "ribonucleic acid" (RNA), and the nucleoprotein is named accordingly. The second type of nucleoprotein contains a desoxypentose. Where this sugar has been identified definitely, it has proved to be D-2-desoxyribose. The corresponding nucleic acid is called "desoxypentosenucleic acid" or desoxyribonucleic acid" (DNA).

In addition to the different sugars, the two types are distinguished by the pyrimidine bases which they contain; while both contain the purines, adenine and guanine, and the pyrimidine, cytosine, RNA in addition contains uracil (pyrimidine), and DNA thymine (pyrimidine).

RNA	*DNA*
H_3PO_4	H_3PO_4
Ribose	Desoxyribose
Adenine	Adenine
Guanine	Guanine
Cytosine	Cytosine
Uracil	Thymine (methyl uracil)

The nucleic acids consist of multiple *nucleotide* units (ribotides and desoxyribotides), each nucleotide containing phosphoric acid, linked to the sugar, which is in turn linked to a purine (purine nucleotide) or a pyrimidine (pyrimidine nucleotide) base. Being acid in nature, these are named, according to their base component, as follows: adenylic, guanilic, cytidilic, thymidilic, and uridylic acids, respectively. Removal of phosphoric acid, by hydrolysis, leaves the corresponding *nucleosides* (base plus sugar), also designated ribosides and desoxyribosides, according to the nature of the sugar component, or, specifically, adenosine, guanosine, cytidine, thymidine, and uridine, respectively.

In the early days of nucleic acid chemistry, RNA was isolated from yeast. It was thought to be restricted to the plant world, and was called "yeast nucleic acid" or "plant nucleic acid." DNA was obtained from fish sperm and thymus gland, and was called "thymonucleic acid" or "animal nucleic acid," in accordance with its assumed distribution in nature.

INTERRELATIONS OF METABOLISM OF NUCLEIC ACIDS WITH OTHER FOODSTUFFS

As illustrated in Figure 20, the nucleic acids and their derivatives are related metabolically to a number of other foodstuffs. However, no obvious connections are found between the metabolism of lipids and that of nucleic acids, aside from the rather nonspecific contribution of lipids to metabolic CO_2.

The pathways of protein metabolism provide much of the raw material for the synthesis of purines and pyrimidines, viz., glycine, formate, (C_1), and "NH_3" for the former, and oxalacetate (from aspartate) and "NH_2" for the latter (in certain cases, formate also). Carbohydrate supplies oxalacetate for the synthesis of pyrimidines, and is also the ultimate source of the pentoses.

The "free" nucleotides, synthesized from the same raw materials as the "bound" nucleotides, exert a considerable influence on the metabolism of all classes of foodstuffs. As collecting, storing, and transferring agents for high-energy phosphate, and as cofactors for biological oxidation and certain other types of reactions (p. 196), the free nucleotides of the tissues may control the rates of many reactions through variations in concentration.

The nucleic acids and nucleoproteins themselves, however, are probably much more important as agents controlling intermediary metabolism, although their exact roles remain speculative. They probably control the genetic pattern of potential enzymes and metabolic pathways in the cell at the nuclear level, and possibly regulate the final realization of that pattern in the cytoplasm. An abnormal variation of these functions may be seen in the mechanism of virus infections.

Thus, it is seen that a reciprocal type of relation exists between the nucleic acids (and derivatives) and other classes of metabolites; the latter provide the raw materials (and probably the energy) for the synthesis of the nucleic acids and derivatives, which in turn exert an influence over the general pattern and rate of metabolism of the other foodstuffs.

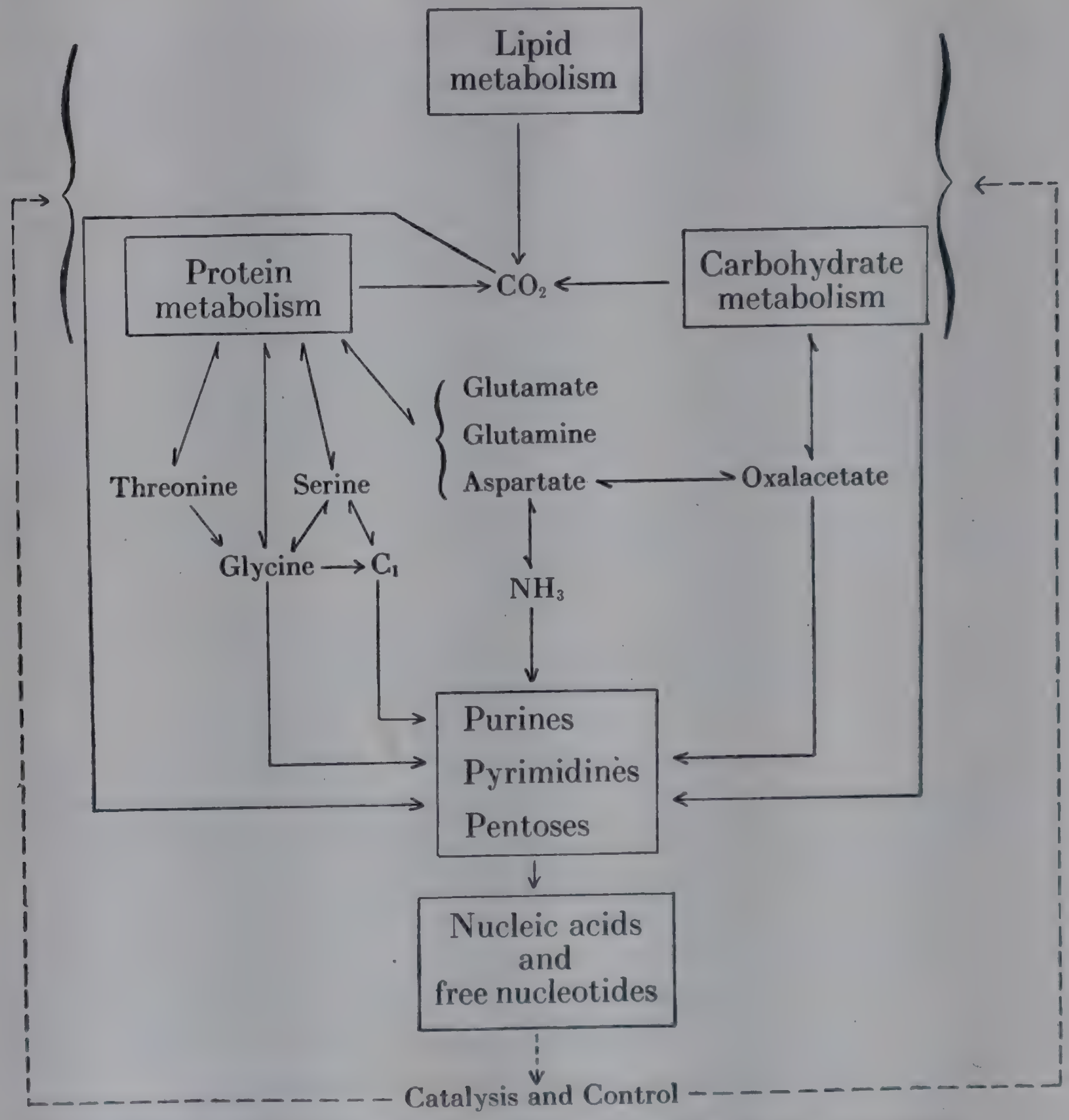
Lipid metabolism
Protein metabolism
CO_2
Carbohydrate metabolism
Glutamate
Glutamine
Aspartate
Oxalacetate
Threonine
Serine
Glycine
C_1
NH_3
Purines
Pyrimidines
Pentoses
Nucleic acids and free nucleotides
Catalysis and Control

Fig. 20. Metabolic interrelations of nucleic acids with other foodstuffs.

BIOLOGICAL SIGNIFICANCE OF NUCLEIC ACIDS

Information accumulating in recent years has stimulated intense interest in the biochemistry of nucleic acids. The intracellular distribution of desoxyribosenucleic acids (DNA) and pentosenucleic acids (RNA) may be outlined as follows: (1) all of the DNA is in the chromosomes in the nucleus; (2) approximately 70 per cent of RNA of the liver cell is in the cytoplasm (50% microsomes, 20% mitochondria); (3) 30 per cent of the RNA is in the nucleus (20% nucleolus, 10% chromosomes). The association of nucleic acids with chromosomes and viruses, and indications of their influence in protein synthesis, suggest that these substances are of great fundamental biological importance. It seems desirable here to point out certain of the directions of current studies in this field, even though they have little direct clinical significance.

Viruses. Until comparatively recently, it was generally agreed that viruses were living organisms characterized, among other things, by: (1) small size (passage through bacterial filters), (2) inability to grow and multiply on artificial culture media, (3) ability to multiply or reproduce within the living cells of the host, and (4) the development of mutation forms during multiplication. Several plant viruses have been shown to be crystallizable nucleoproteins, and many animal viruses are largely if not entirely nucleoprotein. Some contain only desoxyribosenucleoproteins, others (e.g., tobacco mosaic virus) only pentosenucleoproteins, while some contain both. It has been shown that if the crystalline tobacco mosaic virus material, nonliving by usual standards, is introduced into a healthy tobacco plant, the latter develops the disease in typical form. Moreover, the virus nucleoprotein can be subsequently recovered in amounts greatly exceeding that originally introduced, i.e., it reduplicates (reproduces?) with progression of the disease.

Viruses vary widely in size and structural complexity, certain of the smaller ones exhibiting the physical and chemical behavior of molecules, whereas certain of the larger ones approach microorganisms in size and in structural and functional complexity. The observations reviewed above have led to the suggestion that viruses provide a link between molecules and "living" cells, completing the pathway from such simple structures as electrons to the highly complex structure of the mammalian organism.

Viruses possess no enzyme activity (other than virus activity); nevertheless, on entering susceptible cells, they alter and direct certain metabolic sequences within the cell into new channels. Of particular significance is the fact that metabolic precursors are derived from the host cell for construction of the nucleic acid and protein molecules of multiplying virus. These transactions between the host and the virus are apparently irreversible. Synthesis of the host cell nucleic acids may actually cease, while the specific virus nucleic acid continues to accumulate, as in the case of bacteria infected by a bacteriophage, a bacterial virus. This phenomenon may occur without demonstrable alteration in oxygen utilization, i.e., metabolic activity, of the infected cell.

Chromosomes—Genes. Present evidence indicates that chromosomes, of both spermatozoa and somatic cells, consist largely of desoxyribosenucleoproteins. The obvious implication is that their component genes are made up largely of these substances, which, therefore, may be regarded as the "genetic material," concerned in processes of cell reproduction. It is pertinent in this connection that the amount of DNA in spermatozoa containing the haploid number of chromosomes is one-half that of the somatic nuclei of the same species. Moreover, there is a quite striking, although not absolute, uniformity and constancy in the DNA content of the nuclei. The chromosomes are the only cell elements known to contain DNA, but they contain, in addition, a pentosenucleoprotein. It is believed by some that the former is concerned in genetic functions and that the latter participates indirectly in metabolic activities of the cell by making material contributions to the cytoplasm.

Genes resemble viruses in at least two important characteristics: (1) they are autoreproducible, their exact duplication during mitotic division endowing each daughter cell with all of the specific hereditary biochemical potentialities and mechanisms of the original cell; (2) they are capable of undergoing mutation. In determining the biochemical characteristics of the cell, dependent largely on enzymes, it appears that the DNA-proteins are responsible in some way for the synthesis of these substances and that each gene "catalyzes" the production of a single enzyme ("one gene-one enzyme" hypothesis). The manner in which this is accomplished is not known. In certain viruses (e.g., tobacco mosaic), a pure RNA-protein apparently performs this function.

"Transforming Substance" of Bacteria. Certain strains (e.g., Type 3) of pneumococcus possess capsules containing specific polysaccharides and others (e.g., Type 2) do not. This capacity for formation of a type-specific capsule is a hereditary characteristic. If one adds to an appropriate culture of a nonencapsulated strain an extract of an encapsulated strain, the former type is transformed to the latter, developing its characteristic capsular polysaccharide, as do its progeny subsequently. The active agent (transforming factor) seems to be desoxyribosenucleic acid. It apparently endows the bacterial cell with the capacity for synthesizing an enzyme or enzyme system that it did not previously possess, which, in turn, catalyzes the formation of the type-specific capsular polysaccharide. Inasmuch as this new function is subsequently transmissible, this may be regarded, in effect, as an example of induced mutation. Many different specific transforming factors have been found for pneumococci, one for Hemophilus and one for Escherichia coli. All appear to be desoxyribonucleates. It is perhaps of fundamental importance that these agents, as is true of genes and viruses, are reduplicated by the bacterial cell and, like viruses, can be recovered in large amounts from descendants of the cell into which they originally entered.

Role of Nucleic Acids in Mutation and Carcinogenesis. The fact that genes and viruses, largely if not entirely nucleoprotein in nature, possess in common the highly distinctive properties of autoduplication and susceptibility to mutation, raises the fundamentally important question of the relation of nucleic acids to these phenomena. The former has been referred to previously; genes and viruses are the only two sub-cellular substances known to be capable of duplicating ("reproducing") themselves. They also can undergo mutation "spontaneously," and as a result of exposure to certain mutagenic agents. The latter include: (1) ultraviolet light, (2) ionizing radiations (e.g., X and gamma rays and neutrons), and (3) certain chemicals (e.g., colchicine, nitrogen mustards, certain chemical carcinogens, e.g., methylcholanthrene).

Many if not all of these agents are capable of depolymerizing DNA, and it is possible that this is the basis of their mutagenic action. This subject may have basic relevance to the problem of carcinogenesis, which, according to some students of this subject, is one of induced mutation. This opinion is by no means unanimous. It may be significant in this connection, however, that such factors as ultraviolet light, ionizing radiations, and at least some chemicals that are mutagenic, are also carcinogenic. Moreover, certain types of malignancy are known to be due to viruses, which, as indicated elsewhere (p. 193), not only are largely if not entirely nucleoprotein in nature, but also impose upon the invaded cell their own specific nucleic acid synthesizing capacities. These observations suggest that the induction of malignancy may be related fundamentally to the development of an abnormal type of nucleic acid metabolism in the cell.

Role of Nucleic Acids in Protein Synthesis. Growth and maintenance of the organism require continual synthesis of protein molecules, specific for each cell. The mechanism of protein synthesis is not known, but it unquestionably involves enzymes, which are themselves protein in nature. The cytoplasmic enzyme pattern is apparently determined genetically, i.e., by the DNA-containing genes in the nucleus. However, although in this sense the nuclear nucleic acids may be regarded as the ultimate determinants of all metabolic reactions, a more intimate relation to protein synthesis has been suggested by certain observations.

RNA is present in greatest abundance in the cytoplasm of actively growing cells (e.g., embryonic, tumor, tip of plant root) and in cells engaged in the production and excretion of proteins (e.g., pancreatic acinar cells). Increased protein synthesis, e.g., stimulation of pancreatic secretion by pilocarpine, is accompanied by an increase in RNA. Conversely, when production of protein by liver cells is inhibited by starvation, their RNA content decreases simultaneously.

The nucleus apparently contains none of the important enzyme systems concerned in the metabolic activities of the cell, and nucleic acids are the only substances that have been shown to be synthesized in this situation. It would therefore appear, in view of the localization of oxidative and phosphorylating enzyme systems in the mitochondria, and of others elsewhere in the cytoplasm, that the nucleus depends on the latter for its energy supply. Opinion differs as to the relative importance of the nucleus and the cytoplasm in protein synthesis. It is generally agreed, however, although not definitely established, that nucleic acids or nucleoproteins play an important role in this phenomenon. There are indications that the nuclear RNA may be a precursor of the cytoplasmic RNA, contributing to the latter by diffusion through the nuclear membrane. It is believed by some that the RNA may act in the synthesis of proteins by inducing special molecular orientations. According to one formulation, the euchromatin (DNA) of the chromosomes is involved in synthesizing the proteins through which the genes exert their action, whereas the heterochromatin (containing both DNA and RNA) produces the pentosenucleoproteins of the nucleolus. The latter induce the synthesis of cytoplasmic pentosenucleoproteins which are immediately concerned in the synthesis of the ordinary proteins by the cell. On the basis of available data, it may be assumed that although the nucleus is not involved necessarily in the immediate metabolic activities of the cell, the latter are ultimately dependent upon contributions from both the genetic (DNA) and nongenetic (RNA) components of the nucleus. The picture evoked is that of a continuously reciprocal relationship, the nucleus deriving energy from cytoplasmic mechanisms which can be maintained only by replacement through nuclear activities.

Recognition of the fundamental importance of nucleic acids in protein synthesis has led to concentrated efforts to suppress the growth of malignant lesions by interfering with the synthesis of their nucleic acids. Some success has attended the use, in certain acute leukemias, of folic acid antagonists, which act in this manner, i.e., by inhibiting utilization of "formate" for purine and pyrimidine formation.

FREE NUCLEOTIDES OF BIOLOGICAL IMPORTANCE

In addition to the nucleotides which are components of nucleic acids, a number of substances possessing nucleotide structure exist in the free state in the tissues. They have catalytic functions, act in conjunction with enzyme systems, and probably have no direct metabolic connection with nucleic acids.

Adenylic Acid System. This designation is applied to compounds containing adenine and ribose (adenosine), plus one, two, or three phosphate groups, i.e., adenosine mono- (AMP), di- (ADP), and tri-phosphate (ATP), respectively. The latter two serve as carriers and transfer agents for high-energy phosphate groups, a function intimately bound up with the energetics of all living cells (p. 5).

Coenzymes. Certain enzymes require for their function the presence of certain organic compounds, rather loosely attached to, and therefore separable from the enzyme protein. These are called coenzymes, certain of which contain nucleotide units or are structurally related to them. These include diphosphopyridine (DPN) and triphosphopyridine nucleotides (TPN), flavin-adenine dinucleotide (FAD), and coenzyme A, which contain the B vitamins, nicotinamide, riboflavin and pantothenic acid, respectively. These coenzymes are of fundamental importance in biological oxidations, which constitute the core of the energy-producing mechanisms of the body. Both types of nucleic acid have since been found in all living cells, rendering the older terminology obsolete. The term "nucleic" or "nucleo-" is derived from the early erroneous belief that nucleic acids and nucleoproteins occur only in the nuclei of cells. At the present time this term refers to a chemically defined class of substances, irrespective of source or location in nature.

DIGESTION AND ABSORPTION

In the course of digestion of nucleoproteins, their protein components undergo the same changes as other proteins, in the progress of which the constituent nucleic acids (RNA and DNA) are liberated. These are hydrolyzed by the enzymes, ribonuclease and desoxyribonuclease, respectively (nucleodepolymerases) of the pancreatic juice into purine and pyrimidine mononucleotides. Each of these "nucleases" may in reality represent several polynucleotidases of different specificity.

The extent to which further degradation occurs in the lumen of the bowel is not clear. The intestinal mucosa, and also other tissues, contain non-specific phosphatases (alkaline and acid) which hydrolyze the sugar-phosphate bond of mononucleotides ("nucleotidase" action), forming nucleosides (purine and pyrimidine) and phosphoric acid. It is not known whether these enzymes are secreted into the intestinal lumen or whether their presence there is due to exfoliated mucosal cells. In the latter case, the reactions described cannot be regarded as part of the process of digestion, but occur largely intracellularly, during the process of absorption of nucleotides. The same question arises concerning purine "nucleosidases," found in the intestinal fluids, which split purine nucleosides into their pentose and purine base components. There is evidence that this reaction involves phosphorolysis rather than hydrolysis (i.e., "nucleosidase" = nucleoside phosphorylase), which would localize it as an intracellular rather than a digestive process. It appears likely, therefore, that the end-products of nucleic acid digestion are largely if not entirely purine and pyrimidine nucleotides, some question existing as to participation of phosphatases and purine nucleosidases in the process of digestion. These substances are absorbed readily from the small intestine. As indicated above, further degradation of nucleotides may occur within the intestinal mucosal cells, i.e., to nucleosides and phosphoric acid, through the action of phosphatases. Some of the purine nucleosides may also be split here (nucleoside phosphorylase) to purine bases and pentose. Absorption occurs mainly via the portal circulation, the bulk of the products of digestion and intestinal metabolism of nucleic acids, as of proteins, reaching the liver before entering the systemic blood stream.

INTERMEDIARY METABOLISM

For convenience, the metabolism of the nucleic acids may be subdivided into (1) the metabolism of components, (*a*) nonnitrogenous and (*b*) nitrogenous, and (2) the metabolism of the macromolecules. The over-all picture of nucleic acid metabolism is illustrated in Figure 21.

Nonnitrogenous Constituents. Phosphate is readily obtained from the diet and endogenous sources. It is excreted in the urine as inorganic phosphate at the end of its metabolic career.

Ribose and desoxyribose are derived more or less directly from glucose or its metabolites. It is doubtful whether dietary pentoses contribute significantly to the synthesis of nucleic acids. The catabolic pathways of the pentoses are not firmly established, but it is probable that they eventuate in CO_2 and water.

Purines and Pyrimidines. Purines liberated by digestion of nucleic acids, or by metabolic splitting of nucleic acids and nucleotides are apparently largely catabolized directly to waste products, with the exception of a small amount of adenine, which may be incorporated into nucleic acids. Pyrimidine bases are probably not formed in the process of digestion, as indicated above. Pyrimidine nucleosides and nucleotides, exogenous and endogenous, may be utilized for synthesis of nucleic acids.

In view of the relatively slight utilization of preformed purines and pyrimidines by the mammalian organism, biosynthesis of these compounds is of fundamental importance. Ammonia, carbon dioxide, "formate" (one-carbon fragment), and glycine are utilized for the formation of both purine and pyrimidine moieties of nucleotides, the base-sugar-phosphoric acid (i.e., nucleotide) linkages being formed before completion of the purine or pyrimidine ring structure. This apparently accounts for the comparative nonutilization of the preformed bases in this process. Incorporation of "formate" requires participation of folinic acid (citrovorum factor) as a coenzyme. This is one of the fundamental biological actions of the folic acid group of vitamins, and is the basis for many of the manifestations of deficiency in these substances. Pyrimidine nucleosides (i.e., base-sugar) are incorporated in nucleotides to a considerable extent, the complete performed nucleotide linkages not being required as in the case of the purines. Nucleic acids are formed by polymerization of large numbers of purine and pyrimidine nucleotides, the sugar of one being linked to the phosphate of another.

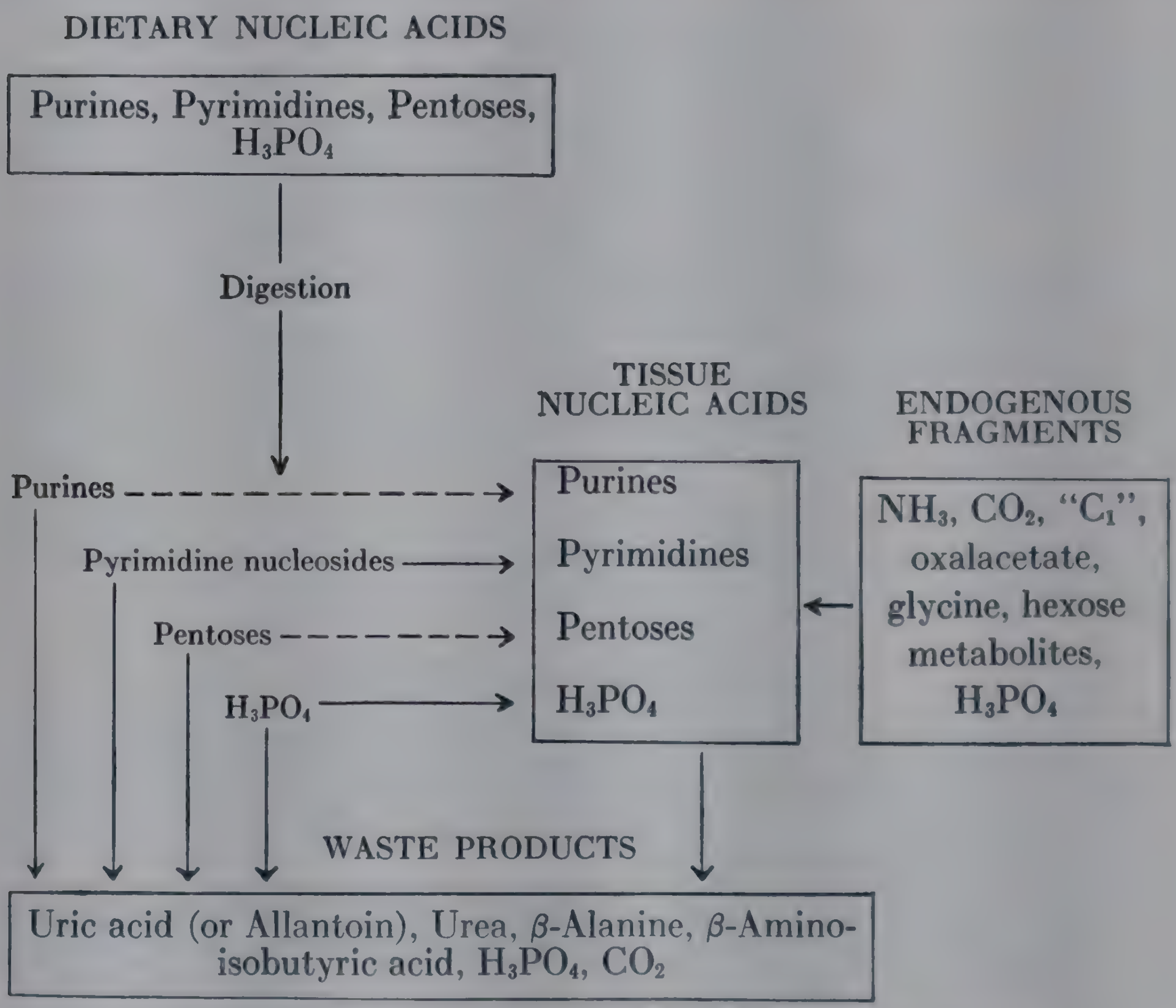
DIETARY NUCLEIC ACIDS
Purines, Pyrimidines, Pentoses, H_3PO_4
Digestion
TISSUE NUCLEIC ACIDS
ENDOGENOUS FRAGMENTS
Purines
Pyrimidine nucleosides
Pentoses
H_3PO_4
Purines
Pyrimidines
Pentoses
H_3PO_4
NH_3, CO_2, "C_1", oxalacetate, glycine, hexose metabolites, H_3PO_4
WASTE PRODUCTS
Uric acid (or Allantoin), Urea, β-Alanine, β-Amino-isobutyric acid, H_3PO_4, CO_2

Fig. 21. Over-all metabolism of the constituents of the nucleic acids.

Nucleic acids (i.e., nucleoproteins) of all cells undergo continual degradation and resynthesis, those of the cytoplasm (RNA) much more actively than those of the nucleus (DNA). Changes in the concentration of RNA in a tissue are generally correlated with the rate of synthesis of cytoplasmic protein, whereas the rate of DNA turnover is correlated with the rate of cell division. The degradation involves depolymerization to mononucleotides, which are then split enzymatically into their component units. The purine bases (adenine and guanine) formed in the course of catabolism of nucleic acids and free nucleotides undergo oxidation to uric acid, which is the chief end-product of purine catabolism in man and the higher apes. Other mammals degrade uric acid to allantoin by means of the enzyme, uricase, which is lacking in primates. The major end-product of pyrimidine catabolism is urea.

Uric Acid. From the practical clinical standpoint, interest is centered particularly in uric acid. Since it is a characteristic metabolite of nucleic acids, capable of quantitative measurement in body fluids, it affords a possible approach to studies of disordered nucleic acid or nucleoprotein metabolism.

The site of its formation in man is not certain. The bone marrow may be a major site, possibly also the muscles, and perhaps the liver (main site in birds). Wherever formed, it passes into the blood stream and is excreted mainly in the urine, with small amounts in sweat, saliva, bile, and intestinal secretions. Despite some contradictory data, there is no unequivocal evidence that uric acid is destroyed in man, apart from the relatively small amount acted upon by intestinal bacteria. Variations in the concentration of uric acid in the blood plasma must therefore be due to variations in the rate of either its formation or its excretion (kidneys). In view of present knowledge of the extensive biosynthesis of purines from simple nitrogenous (i.e., ammonia and glycine) and carbon (i.e., CO_2 and "formate") compounds, it is obvious that the ultimate production of uric acid may be influenced by factors that affect the metabolism of protein, carbohydrate, and fat, as well as those affecting the degradation of exogenous and endogenous nucleic acids and nucleotides.

Under conditions of normal production and removal, the body contains a "readily miscible uric acid pool" of about 1.2 grams. About 50 to 75 per cent of this (700-850 mg.) is turned over daily, representing the rate of formation and disposal of uric acid in normal subjects.

BLOOD URIC ACID. The normal uric acid content of whole blood is 2 to 4 mg./100 cc., that of plasma or serum 2 to 6 mg./100 cc. The latter values are much more significant clinically than the former; all determinations should be made in plasma or serum. The blood uric acid is normally affected but slightly, if at all, by ingestion of purine-rich foods. It bears no direct relation to the level of urea or of total NPN (to which it contributes).

URINE URIC ACID. The quantity of uric acid excreted in the urine varies with the purine (nucleoprotein) intake. On a diet very low in nucleoprotein, normal adults excrete 0.2 to 0.5 gm. daily, representing mainly the catabolism of endogenous nucleic acids and nucleotides. The amount varies also with the protein and caloric content of the diet. A high protein and caloric intake results in an increase, and a low protein and caloric intake in a decrease in urine uric acid. On a high purine diet (meat, liver, kidney, sweetbreads, leguminous vegetables) the output may rise to 2 gm. daily; on a mixed diet the average daily excretion is about 0.7 gm. Caffeine and other methylpurines (in tea, coffee, cocoa) cause little or no increase, since they are either largely transformed to urea or destroyed in the organism. During the first few days of life the urine contains relatively large quantities of uric acid, constituting 7 to 8 per cent of the total nitrogen (1–2% in adults). This may lead to the development of "urate infarcts" in the kidneys of infants, due to the relative insolubility of uric acid in an acid medium.

About 90 per cent of the uric acid that enters the glomerular filtrate is reabsorbed in the renal tubules, the normal urate clearance being about 7 to 16 ml./min. This extensive reabsorption is accomplished by an active transport mechanism, a fact of importance in connection with the action of certain uricosuric agents. Such substances as salicylates (large doses), cinchophen, carinamide and Benemid, which greatly increase urinary elimination of uric acid, produce this effect by inhibiting its reabsorption in the renal tubules, presumably by blocking the enzymatic transport mechanism. The uric acid clearance increases accordingly under the influence of these agents, and the blood level may fall (e.g., in gout). The action of carinamide and Benemid is of particularly interest in this connection, since they also block tubular excretion of certain substances (e.g., penicillin, p-aminosalicylic acid, p-aminohippurate, PSP, and Diodrast), and have been employed for the purpose of maintaining therapeutically effective plasma levels of penicillin and p-aminosalicylic acid for prolonged periods. ACTH and the adrenocortical oxysteroids (p. 424) also increase the urinary excretion of uric acid. It is not certain whether this is due primarily to inhibition of renal tubular reabsorption or, in part, to stimulation of purine catabolism. Inasmuch as there is no simultaneous change in creatinine excretion, it has been suggested that determination of the extent of rise in the urinary uric acid: creatinine ratio following administration of ACTH may be useful as a test for the reserve functional capacity of the adrenal cortex (p. 441).

Determination of uric acid excretion in disease is of little clinical importance. Changes in gout are indicated elsewhere (p. 201). It may be increased in patients with leukemia (especially chronic myeloid) and polycythemia, presumably because of increased rate of turnover of blood cell nucleic acids. It may also increase during early periods of remission in pernicious anemia, especially if induced by specific therapy (liver extracts, vitamin B_{12}).

ABNORMAL PLASMA URIC ACID. (1) *Decreased Excretion.* Increased values are observed in all forms of nephritis with nitrogen retention. Retention of uric acid frequently, although not invariably, precedes that of urea. Values as high as 10 mg./100 cc. are frequently observed; figures above 15 mg. are unusual. Administration of purine-rich foods in these conditions may produce an elevation in plasma (or serum) uric acid; this does not occur in normal subjects.

Hyperuricemia may occur, in association with other urinary constituents, in conditions associated with urinary obstruction (prostatic, bilateral ureteral calculus, etc.), urinary suppression (lower nephron nephrosis, necrotizing nephroses, shock, intestinal obstruction, etc.), destructive renal lesions (tuberculosis, pyelonephritis, hydronephrosis, polycystic kidney, etc.), renal hypogenesis, and congestive heart failure.

Increase in plasma uric acid may occur in eclampsia, in the absence of retention of urea. It has been suggested that in this condition there is a specific decrease in uric acid clearance, with no associated abnormality in other functions of the kidney. On this basis, the simultaneous presence of decreased uric acid clearance and normal urea clearance may aid in differentiating true eclampsia from glomerulonephritis complicating pregnancy.

(2) *Hematological Disorders.* The blood uric acid increases commonly in chronic leukemia, particularly myeloid, and also occasionally in polycythemia and multiple myeloma. This is probably a reflection of the greatly increased production of uric acid resulting from the enormous increase in the number of short-lived cells, i.e., increase in nucleic acid turnover and purine catabolism. A similar mechanism probably operates to produce the increase in blood uric acid which occurs early in the course of remissions in pernicious anemia, usually preceding by a day or more the increase in reticulocytes. Renal involvement may contribute to the increase in multiple myeloma.

(3) *Chronic Lead Poisoning.* Hyperuricemia is observed rather frequently in chronic lead poisoning. Although renal damage may be a factor in some cases, in many the cause is not apparent. Gout ("saturnine gout") may occur as a complication of chronic plumbism.

(4) *Hepatic Disease.* In the dog, hepatectomy is followed by an increase in uric acid in the blood and urine. This led to the view that the liver is an important site of destruction of this substance, at least in the dog (conversion to allantoin). However, more recent studies have failed to produce conclusive evidence of significant degradation of uric acid in man, although slight elevations of blood uric acid have been reported occasionally in patients with hepatic disease. In fact, low values have been observed in severe cases of acute hepatic necrosis, suggesting the possibility that the liver may be one site of normal production of uric acid in man; it is the main site in birds.

(5) *Gout.* This disease, of unknown etiology and as yet uncertain pathogenesis, is characterized by a derangement of purine metabolism, the most valuable reflection of which, from the standpoint of early diagnosis, is elevation of the plasma uric acid concentration. Hyperuricemia not attributable to such causes as renal failure, leukemia, polycythemia, multiple myeloma, etc., should be regarded as highly suggestive of gout, until proven otherwise.

Increase in plasma (serum) uric acid, often as high as 10 mg. and occasionally 15 mg./100 cc., occurs practically invariably in acute attacks of gout. Some degree of elevation usually persists between attacks but it may be intermittent and therefore missed. Hyperuricemia has been found in 25 per cent of blood relatives, themselves without symptoms, of gouty subjects, values in women in this category being somewhat lower than in men.

Despite the rather consistent presence of hyperuricemia in acute gout, the elevated blood uric acid level is apparently not the cause of the acute symptoms. There is no consistent change in the plasma level nor, as erroneously stated in the older literature, in urinary excretion of uric acid. No such attacks occur in patients with renal failure with high blood uric acid concentrations. Moreover, there is no consistent quantitative correlation between the clinical response to such agents as ACTH, cortisone, cinchophen, salicylates, carinamide, or Benemid, and their uricosuric or plasma urate-lowering effects. Remissions induced by colchicine are not accompanied by significant change in uric acid in the plasma or urine. Administration of uric acid, orally or intravenously, does not precipitate an attack, even in gouty subjects. Observations such as these have led to the view that some purine metabolite, other than uric acid, probably a precursor of the latter, is the agent directly responsible for the acute attack.

The increased concentration of urate in the body fluids is, of course, the direct cause of the local tissue deposits in chronic gout. Because of their comparatively low solubility at the usual pH of the tissues, urates precipitate from the tissue fluids and are deposited particularly in the kidneys, skin, subcutaneous tissues, cartilage, bursae, tendons, ligaments, synovial membranes, and sclerae. Acting as foreign bodies, these deposits incite an inflammatory reaction. Those that are very superficial are recognizable as tophi; if close to bones, they cause erosions that are demonstrable roentgenographically. Deposits in the kidneys may result in inflammatory lesions of the glomeruli with impairment of renal function, a common observation in chronic gout and a common cause of death in this condition. Urate urinary calculi occur rather frequently (10–15%).

It has been found that the "urate pool," i.e., the amount of urate capable of mixing promptly with intravenously injected urate, is markedly increased in subjects with gout. Values 15 to 30 times the normal quantity (1.2 gm). have been observed. Inasmuch as there is no satisfactory evidence that uric acid is destroyed in man, and no indication of impaired urinary excretion of uric acid in uncomplicated gout, the view is gaining ground that the demonstrated accumulation of this substance in this disease is probably due to increased production. Recent demonstration of an increased rate of formation of uric acid from glycine in a patient with gout lends support to this view. The nature of the underlying mechanism is not known. However, in the case of gout occurring as a complication of leukemia and polycythemia, increased metabolic turnover of nucleic acids probably plays a causative role.

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CHAPTER V

Metabolism of Hemoglobin and Porphyrins

Hemoglobin is a conjugate of a protein, *globin,* and four molecules of *heme,* a substance which is responsible for the characteristic color and oxygen-carrying properties of hemoglobin. Heme is a type of porphyrin (protoporphyrin) to which is attached an atom of iron in ferrous form (ferroprotoporphyrin).

Porphyrins are complex cyclic compounds composed of four pyrrole units $[(\cdot CH{:}CH)_2 : NH]$ linked by methyne ($-CH=$) bridges (i.e., cyclic tetrapyrroles) to form a large ring structure. The protoporphyrins contain certain side-chains attached to carbon atoms in the pyrrole rings, viz., methyl, vinyl, and propionic acid groups. Because of the many possible differences in arrangement of the side-chains, there are fifteen theoretically possible protoporphyrin isomers, only one of which (protoporphyrin III) is found in nature.

In addition to protoporphyrin (in heme), two other types of porphyrin are of biological importance in man, i.e., uroporphyrins (acetate and propionate side-chains) and coproporphyrins (methyl and propionate side-chains). Of the four theoretically possible isomers of each of these, only two occur in nature, types I and III. The prefixes, "uro" and "copro," indicate the medium from which each of these compounds was originally isolated, but it is now known that both may be excreted in both the urine and the feces.

The protoporphyrins are able to form complexes with iron, forming heme, the prosthetic group of a class of hemoproteins which are concerned directly or indirectly in facilitating the reaction which is the basis of practically all bioenergetics, i.e., the union of oxygen and hydrogen to form water. These hemoproteins include hemoglobin, myoglobin (muscle hemoglobin), cytochromes, catalases, and peroxidases.

Hemoglobin is the carrier of atmospheric oxygen from the lungs to the tissue cells. It also participates in the transport of carbon dioxide (p. 359) and in the mechanism of regulation of acid-base balance (p. 326). The oxygen may be stored temporarily in muscle, attached to myoglobin. The combination of the oxygen from the air with the hydrogen of metabolic substrates to form water is catalyzed by the cytochromes and cytochrome oxidase. Ancillary roles in biological oxidations are played by the catalases and peroxidases, other heme-containing enzymes. The vital importance of the hemoproteins is indicated by the fact that inhibition of the oxygen transport system (hemoglobin) by carbon monoxide (p. 209), or inhibition of the oxygen utilization system (cytochromes) by cyanide, results in rapid death of the organism. It is significant, too, that chlorophyll, a substance involved in the photosynthetic reactions of plants, is a magnesium-porphyrin which catalyzes the cleavage of water into hydrogen and oxygen. Evidently, derivatives of porphyrins are of wide significance in comparative biochemistry, from the initial fixation of solar energy in green plants to its eventual utilization by man.

Synthesis of Porphyrins. The complex porphyrin ring is synthesized from small units, i.e., glycine, succinic acid, and a one-carbon fragment ("formate") derived from glycine, the latter forming the methyne bridges that link the four pyrrole rings. It seems probable that the first porphyrin formed is uroporphyrin (acetate and propionate side-chains), mainly Type III and a relatively small amount of Type I. Decarboxylation of the acetate side-chains to methyl groups results in coproporphyrins (methyl and propionate side-chains) Types I and III. Coproporphyrin I, representing the minor metabolic pathway, is not utilized in the organism, and all that is formed is excreted in the urine (35–225 μg. daily, av. 100 μg.) and feces (via the bile) (200–1000 μg. daily, av. 600 μg.). The major metabolite, coproporphyrin III, is formed in amounts approximating 300 mg. daily. All but about 0.2 mg. is converted to protoporphyrin III by transformation of two of the propionate side-chains to vinyl radicals. The approximately 200 μg.

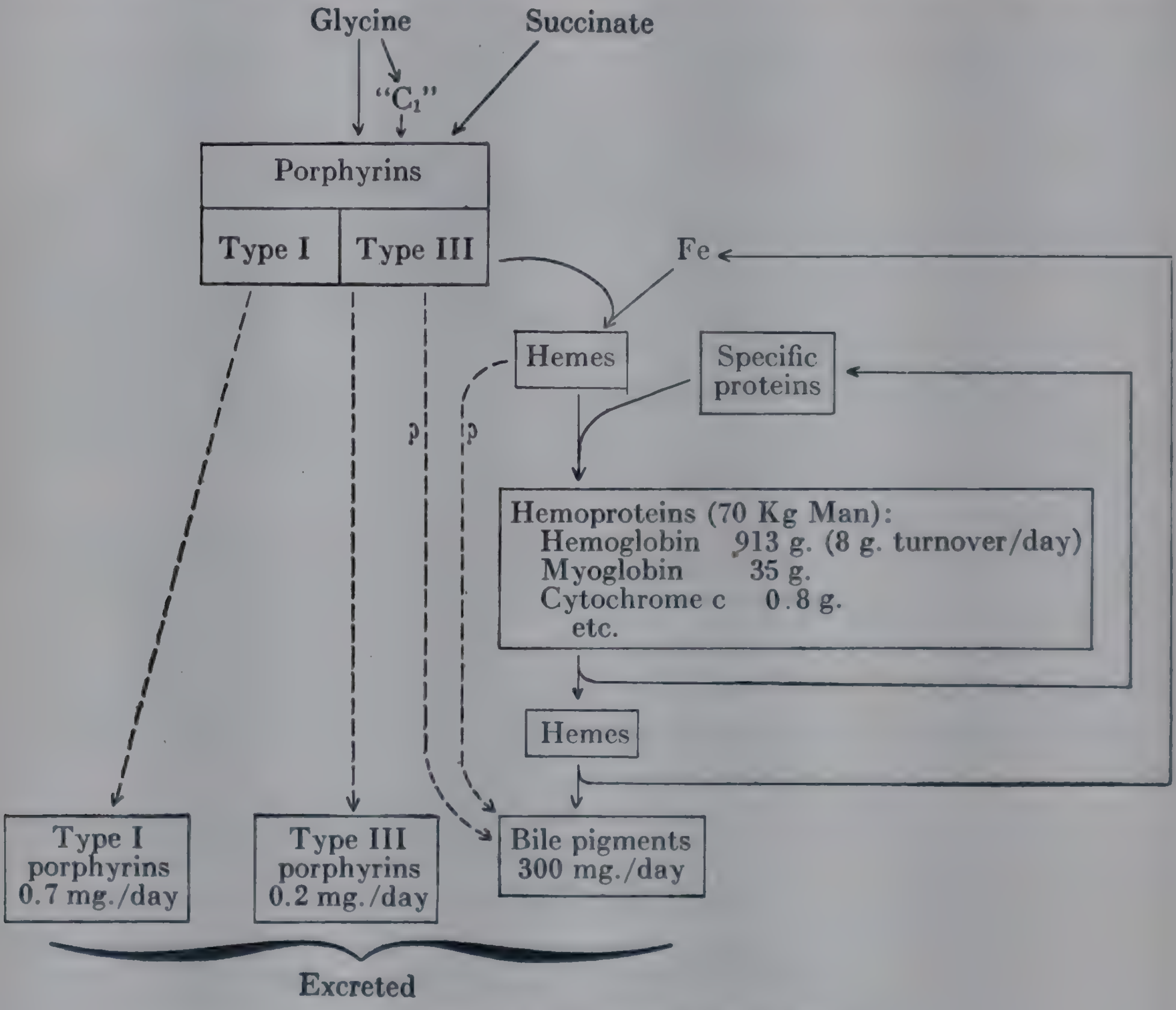
Glycine
Succinate
"C_1"
Porphyrins
Type I
Type III
Fe
Hemes
Specific proteins
p
p
Hemoproteins (70 Kg Man):
Hemoglobin 913 g. (8 g. turnover/day)
Myoglobin 35 g.
Cytochrome c 0.8 g.
etc.
Hemes
Type I porphyrins 0.7 mg./day
Type III porphyrins 0.2 mg./day
Bile pigments 300 mg./day
Excreted

Fig. 22. Over-all metabolism of porphyrins.

that escapes this conversion is excreted in the urine (50 μg. daily) and feces (via the bile) (150 μg. daily). Uroporphyrins are not found in the urine and feces under normal conditions, but are excreted in certain forms of porphyrinuria (p. 210). It should be noted that although Type I porphyrins are formed in much smaller quantities than Type III (0.7 mg. vs. 300 mg. daily), because of the non-utilization of the former in the body in contrast to the almost complete conversion of the latter to protoporphyrin, and eventually to heme, Type I coproporphyrin excretion (0.7 mg. daily) normally exceeds that of Type III (0.2 mg. daily).

Synthesis of Hemoglobin. Insertion of an atom of ferrous iron into protoporphyrin III forms heme. This is then coupled to various proteins to produce the conjugated hemoproteins, such as hemoglobin, myoglobin, cytochrome c, catalases, and peroxidases. Certain hemoproteins, e.g., cytochrome oxidase, contain porphyrins other than protoporphyrin, but these are probably derived from the latter.

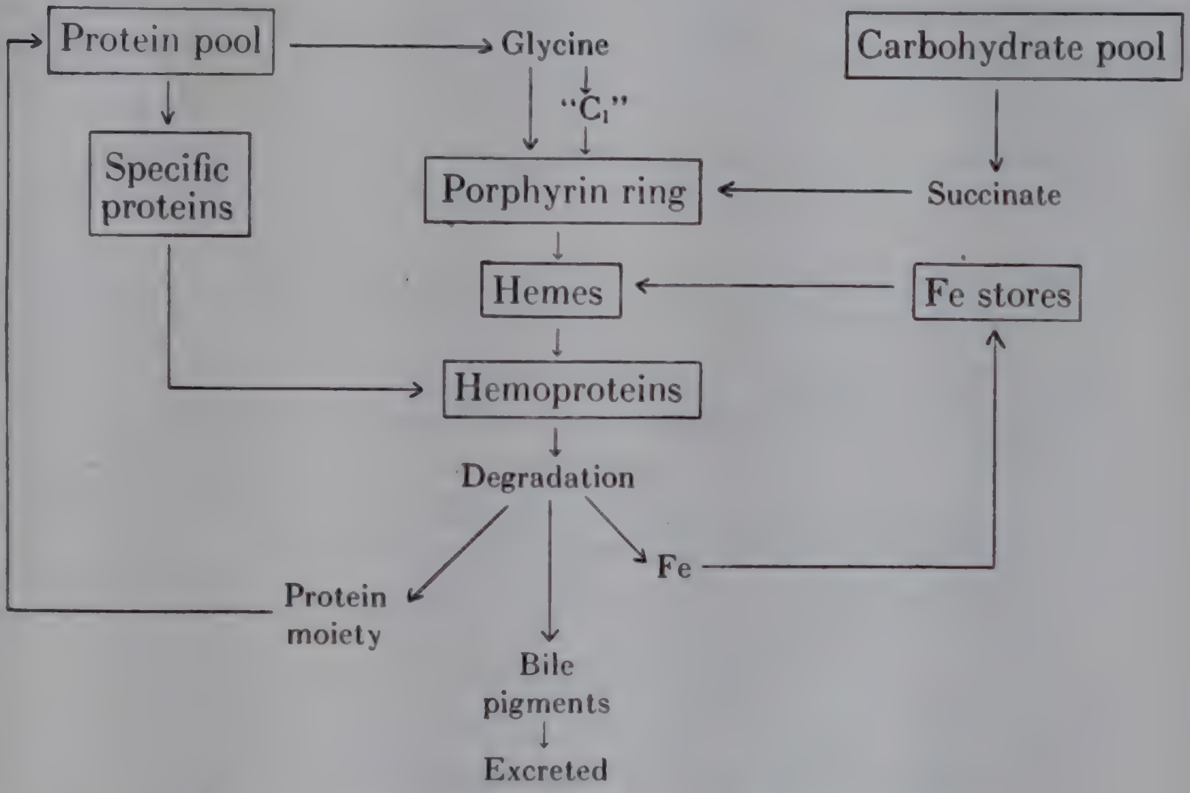

Fig. 23. Interrelations of porphyrins with other metabolites.

From the clinical standpoint, interest is centered particularly in the synthesis of hemoglobin, which, in the adult, is restricted normally to the red bone marrow. Three components are required: (1) protoporphyrin III, synthesized as indicated above; (2) globin, produced by the usual mechanisms of protein synthesis; and (3) iron, the sources and metabolism of which are considered elsewhere (p. 257). In addition to the actual constituents of hemoglobin, certain cofactors are required, viz., the folic-folinic acid group of vitamins, vitamin B_{12} ("extrinsic factor") and the "intrinsic factor" necessary for its absorption, and copper. The requirement for folinic acid and vitamin B_{12} may be due to their role in the utilization of a one-carbon ("formate") fragment in porphyrin synthesis. No satisfactory explanation can be offered for the role of copper. Synthesis of hemoglobin proceeds concomitantly with maturation of the erythrocyte, rendering a decision difficult as to whether a given cofactor is concerned in one process or the other. In adults (70 kg. man), approximately 8 gm. of hemoglobin are synthesized (and degraded) daily, corresponding to about 300 mg. of porphyrin (4 per cent of hemoglobin molecule).

The primitive red cells contain porphyrins rather than hemoglobin. As the cell matures, the content of porphyrin decreases and that of hemoglobin increases, these changes being reflected in alterations in the staining properties of the cell, i.e., change from basophilia to acidophilia. Normal erythrocytes contain small amounts of protoporphyrin and traces of coproporphyrin, mainly Type III. The quantity of protoporphyrin increases in certain conditions in which hemoglobin synthesis is inhibited, e.g., iron deficiency. Increases have been observed also in lead poisoning and hemolytic anemias. Low or normal values are found in pernicious anemia. Increase in coproporphyrin has been observed in conditions accompanied by reticulocytosis, i.e., increased rate of erythropoiesis, e.g., in lead poisoning and excessive hemolysis, and at the onset of remissions in pernicious anemia.

Catabolism of Hemoglobin. Within the mature erythrocyte, hemoglobin undergoes no alteration during the "lifetime" of the cell, about 120 days. Upon disintegration of the cell, the hemoglobin liberated, amounting to about 8 gm. daily, undergoes a sequence of changes, as a result of which its iron and globin components are returned to the general community of metabolites, the former being virtually completely reutilized for resynthesis of hemoglobin (and other iron-heme compounds), the latter following the pathways of protein catabolism. The protoporphyrin component is not utilized. It undergoes a series of changes which eventuate in the formation of bilirubin, which enters the blood stream, is removed by the liver, excreted in the bile and converted, in the intestine, to the end-products of the degradation of heme, i.e., the bilinogens and bilins. These are excreted largely in the feces, but also in the urine (after reabsorption from the intestine).

These aspects of hemoglobin catabolism are considered in detail elsewhere (p. 516), as they are of particular importance clinically in relation to the study of liver function.

"Abnormal" Hemoglobins. Attention has been directed to the presence, especially in sickle cell anemia, and in subjects possessing the sickle cell trait, of types of hemoglobin that differ from that present in normal adults. At least four such forms have been found, designated hemoglobins C, D, F, and S, respectively, differing from the normal (Type A) in electrophoretic pattern, and, in certain instances, in the oxygen dissociation curve and susceptibility to denaturation by alkali (hematin formation).

Fetal hemoglobin (F) comprises about 80 per cent of the total hemoglobin in the newborn. It takes up oxygen more readily at low oxygen tensions and releases carbon dioxide more readily than adult hemoglobin (A). It is therefore admirably adapted to the conditions of relatively low oxygen tension in the fetus.

At birth, efficient gas exchange in the lungs replaces the relatively inefficient placental mechanism, and the fetal type of hemoglobin begins to decrease, disappearing from the circulation at the end of 4 to 7 months. Hemoglobin F is more resistant to denaturation by alkali and is more susceptible to conversion to methemoglobin by nitrites. The susceptibility of young infants to develop methemoglobinemia, with consequent cyanosis and anoxia, after exposure to nitrites (contaminated water), has been attributed to this cause.

A type of hemoglobin not present in normal subjects (hemoglobin S) predominates (about 80 per cent of total hemoglobin) in patients with sickle cell anemia, a hereditary disorder. It comprises 20 to 45 per cent of the total hemoglobin in subjects exhibiting only the sickle cell trait (sickling only *in vitro*, at lowered oxygen tensions). Smaller amounts of other types of hemoglobin (C and D) have also been found in this condition. The reduced form of hemoglobin S is much less soluble than normal reduced hemoglobin. Accordingly, it has been suggested that red cells containing a large proportion of their hemoglobin in this form undergo sickling at normal venous oxygen tensions as a result of crystallization of this substance ("tactoid" formation). The anemia, hemolysis, and thrombosis which characterize this disease are attributable to the increased fragility and distortion of these cells. If smaller amounts of the hemoglobin are in these abnormal forms (S, C, D), sickling does not occur at oxygen tensions present in the blood, but can be induced *in vitro* by exposing the cells to unphysiologically low oxygen tensions (i.e., sickle cell trait). In some instances, at least, the difference between these two states is explicable on the basis of Mendelian inheritance, sickle cell anemia occurring in homozygous SS individuals (all hemoglobin type S) and the sickle cell trait in heterozygous SA individuals (hemoglobin half type S and half type A). This explanation does not suffice in all cases.

HEMOGLOBINEMIA

Hemoglobinemia (hemoglobin free in the plasma) may occur when excessive numbers of erythrocytes are destroyed very rapidly, particularly when the hemolytic process occurs intravascularly. The condition is accompanied by anemia, reticulocytosis, an increase in serum bilirubin and, at times, methemoglobinemia. If the concentration of hemoglobin in the plasma exceeds 60 to 150 mg. per 100 cc., hemoglobinuria may occur (p. 178), and may persist until the plasma Hb has fallen to 30 to 50 mg. per 100 cc. Excessive amounts of urobilinogen are excreted in the urine and feces (pp. 529, 531).

Hemoglobinemia therefore is a manifestation of severe hemolytic anemias, especially the acute variety, but also exacerbations of chronic forms. The following factors may be active in bringing about this condition:

(*a*) *Bacteria.* Hemolytic streptococcemia, yellow fever, severe typhoid and scarlet fevers, clostridia infections.

(*b*) *Protozoa.* Malaria and blackwater fever and Bartonella infection (Oroya fever).

(*c*) *Chemical Agents.* Poisoning with arseniuretted hydrogen, arsphenamines, pyrogallic acid, phenylhydrazine, saponin, sulfonamides and, rarely, lead. Some unchanged hemoglobin may appear in the plasma at times in poisoning with subsubstances that cause methemoglobinemia.

(*d*) *Vegetable Poisons.* Poisoning with ricin, crotin, and poisonous toadstools; favism, due to hypersensitivity to the fava bean.

(*e*) *Animal and Endogenous Poisons.* Poisoning with certain snake venoms and occasionally after severe, extensive burns, administration of serum for tetanus, diphtheria and meningococcus meningitis.

(*f*) *Miscellaneous.* Transfusion with incompatible blood, paroxysmal hemoglobinuria (cold hemolysins and syphilis), paroxysmal nocturnal hemoglobinuria (Marchiafava-Micheli), acute hemolytic anemia of unknown etiology (Lederer's anemia), acute crises in congenital hemolytic jaundice (spherocytic anemia), march hemoglobinuria (strenuous exertion, lordosis), and occasionally after severe intra-abdominal hemorrhage.

ABNORMAL HEMOGLOBIN DERIVATIVES

Certain derivatives of hemoglobin are of clinical importance because of their production and presence in the blood in abnormal amounts in various disease states. In the majority of instances they result from abnormal exposure to toxic chemical agents.

Methemoglobin. This is an oxidized hemoglobin (oxidized heme), differing from oxyhemoglobin (oxygenated hemoglobin) in that it contains ferric iron in contrast to ferrous iron in the latter and that its oxygen is in firm combination and cannot be removed by exposing the blood to a vacuum. Methemoglobin is consequently incapable of functioning as an oxygen-carrier (p. 362). Cyanosis usually develops when the concentration reaches 3 gm. per 100 cc. of blood.

Methemoglobin is present in normal erythrocytes, comprising up to about 1 per cent (average 0.4%) of the total hemoglobin. It apparently exists in reversible equilibrium with hemoglobin, the relative proportions of the two substances being regulated by various reducing and oxidizing substances and enzymes in the red cells. Under normal conditions, of course, this equilibrium is shifted far in the direction of hemoglobin. When the methemoglobin content increases, the cells at first usually remain intact. With very high concentrations, however, the cells may be injured and destroyed and methemoglobin, often with hemoglobin, may be liberated into the plasma and excreted in the urine. The causes of methemoglobinemia may be classified as follows:

(*a*) *Drugs and Other Toxic Agents.* These include nitrites, chlorates, permanganates, aniline, acetanilid, acetphenetidin, antipyrine, nitrobenzene, pyrogallol, sulfonal, plasmochin, methylene blue, and certain sulfonamides.

(*b*) *Other Hemolytic Agents.* Among conditions falling under this heading are congenital hemolytic icterus (acute crises), paroxysmal hemoglobinuria, infection with anaerobic bacteria (clostridia), blackwater fever, and excessive doses of phenylhydrazine.

(*c*) *Enterogenous Cyanosis.* In some cases so classified the production of methemoglobinemia is believed to be due to excessive production and absorption of nitrites from the intestine. These nitrites may be elaborated by nitrite-producing bacteria. Methemoglobinemia has been reported in young infants (under 6 months of age) as a result of ingestion of well-water containing excessive amounts of nitrites (from vegetation). This has been attributed to the unusual susceptibility of the fetal type of hemoglobin to methemoglobin formation by nitrites (p. 207).

(*d*) *Idiopathic.* This is an unusual condition in which cyanosis (methemoglobinemia) is constant and unaffected by therapy. Such cases may be confused with congenital heart disease. It is a congenital condition, in which methemoglobin comprises 30 to 45 per cent of the total hemoglobin.

Sulfhemoglobin. Reduced hemoglobin combines with hydrogen sulfide to form sulfhemoglobin. Sulfhemoglobinemia is an unusual condition which occurs chiefly as a result of the action of nitrites and coal tar preparations (aniline, acetphenetidin, acetanilid) in the presence of excessive amounts of sulfur. It has also been observed in subjects with marked constipation, in the presence of nitrite-producing bacteria in the intestine. Some believe that aniline may "sensitize" hemoglobin so that it unites with sulfides. This condition has been produced in splenectomized animals by feeding large amounts of sulfur without additional medication. Sulfhemoglobinemia has been reported in patients receiving sulfonamides. As is the case in methemoglobinemia, sulfhemoglobin is usually contained in the erythrocytes, but it may occasionally be liberated into the plasma. Cyanosis usually occurs when the concentration of sulfhemoglobin reaches 3 to 5 gm. per 100 cc. of blood.

Carboxyhemoglobin. Carbon monoxide combines with the same group in the hemoglobin molecule as does oxygen, the combining capacity of hemoglobin for both being identical. However, the affinity for carbon monoxide is more than 200 times as great as for oxygen, and in the presence of relatively small concentrations of CO in the air a considerable quantity is taken up by the blood, with consequent reduction in the amount of hemoglobin available for transport of oxygen to the tissues. The dissociation of oxyhemoglobin is also diminished. Carboxyhemoglobin has a bright, cherry-red color. It is formed usually as a result of excessive exposure to artificial illuminating (but not natural) gas, and to automobile exhaust gases, in closed or poorly ventilated rooms.

Hematinemia. Hematin is a ferric complex of protoporphyrin, formed in vitro by treating hemoglobin with dilute acid or alkali. It appears in the blood plasma (hematinemia) under certain abnormal circumstances, particularly in the simultaneous presence of excessive hemolysis and liver damage, usually accompanied by hyperbilirubinemia. This condition has been observed in gas bacillus and other septicemias, severe malaria (black-water fever), severe pernicious anemia, hemolytic anemias, nocturnal hemoglobulinuria, acute hepatic necrosis, and, occasionally, in lead poisoning, and after excessive amounts of phenylhydrazine, acetanilid, and aromatic nitro compounds. According to some, hematin does not occur in the plasma in the free state, but is combined with albumin, forming "methemalbumin" (methemalbuminemia).

Hematinemia unaccompanied by hyperbilirubinemia may occur, occasionally, in conditions in which relatively large amounts of blood are extravasated into epithelial-lined spaces in close communication with the blood stream, e.g., in the kidney. In contact with epithelial cells, hemoglobin is converted to hematin, not to bilirubin.

PORPHYRIA AND PORPHYRINURIA

Conditions in which excessive amounts of porphyrins are excreted in the urine (porphyrinuria) or feces, or both, may be placed in two main categories, (1) congenital (porphyrias) and (2) acquired. The characteristic biochemical features of these conditions are indicated in Table 6. Certain generalizations may be made concerning the basic mechanisms which underlie these abnormalities.

1 Formation and excretion of excessive amounts of type I porphyrins usually result from a greatly accelerated rate of synthesis of heme, e.g., in "erythropoietic" porphyria and hemolytic anemias.

2. Uroporphyrins are excreted only in the porphyrias (i.e., congenital, not acquired porphyrinurias).

3. Accumulation and excretion of excessive amounts of type III porphyrins result from inability to convert them adequately to heme, i.e., interference with heme synthesis.

4. In the absence of an "inborn error" in porphyrin metabolism, increased amounts of coproporphyrin I may appear in the urine in the presence of impaired liver function or biliary obstruction, as a result of diversion of its excretion from the bile to the urine. Under these circumstances, the amount in the feces is correspondingly diminished.

Porphyria. This designation is applied to conditions characterized by excessive production and excretion of porphyrins and/or a precursor, "porphobilinogen." They are apparently congenital in nature, often exhibit a marked familial incidence, and are included among the so-called "inborn errors of metabolism." The nature and time of appearance of the striking clinical manifestations differ in different forms. They include, characteristically: cutaneous photosensitivity, with blistering of exposed surfaces, and subsequent ulceration, scarring and mutilation; a peculiar purplish complexion; gastrointestinal and other abdominal complaints; nervous and mental symptoms. The urine has a pink or red color, which intensifies on standing, exposed to light. It may not be apparent in freshly voided urine. The condition may remain latent for years, and may become manifest as the result of some inciting factor, e.g., alcoholism, barbiturates, or hepatic or some other intercurrent disease.

The abnormalities in porphyrin metabolism in the porphyrias (Table 6) may be outlined as follows:

1. Uroporphyrin I is excreted in the urine. This does not occur in the acquired types of porphyrinuria.

2. In the comparatively rare "erythropoietic" form, excessive amounts of Type I porphyrins are excreted in both urine and feces, uroporphyrin predominating in the former, coproporphyrin in the latter. Increased amounts are present in the bone marrow.

3. In the more common "hepatic" forms, both urine and feces contain excessive amounts of coproporphyrin III. In addition, uroporphyrin I, a "uro-like"

porphyrin III, and "porphobilinogen," probably a porphyrin precursor, may be excreted in the urine. The uroporphyrin is present mainly as a zinc complex, whereas in the erythropoietic form it is mainly in the free state. Increased quantities of porphyrins are present in the liver, but not in the bone marrow. Liver disease and/or hepatic functional impairment, with or without jaundice, can be demonstrated in virtually all cases.

Acquired Porphyrinurias. The various types of conditions in which excessive amounts of porphyrins are excreted in the urine as a result of purely acquired disease are indicated in Table 6. Porphyrinuria as a manifestation of hepatic and biliary tract disorders is considered in detail elsewhere (p. 534). It will suffice here merely to outline certain characteristic features of the conditions in this category.

Table 6. Porphyrinurias

GENERAL TYPE	SPECIFIC CONDITIONS	CHIEF URINARY PORPHYRINS	OTHER CHARACTERISTICS
Hereditary	Erythropoietic porphyria	Uroporphyrin I Coproporphyrin I	Increased porphyrins in bone marrow
	Hepatic porphyria	Coproporphyrin, mainly III. Uroporphyrin I. "Uro-like" porphyrin III	Increased porphyrins in liver. Porphobilinogen in urine.
Hereditary or acquired?	"Mixed," chronic porphyria	Coproporphyrin mainly III. Uroporphyrins as above	Increased porphyrins in liver. Porphobilinogen in urine
Acquired	Toxic agents: Chemicals Heavy metals Acute alcoholism Cirrhosis in alcoholics	Coproporphyrin III	
	Certain liver diseases: Infectious hepatitis Cirrhosis in non-alcoholics	Coproporphyrin I	
	Certain blood dyscrasias: Hemolytic anemias Pernicious anemia Leukemia	Coproporphyrin I	
	Obstructive jaundice	Coproporphyrin I	
	Miscellaneous conditions: Poliomyelitis Aplastic anemias Hodgkin's disease	Coproporphyrin III	

1. Impairment of hepatic excretory capacity, whether due to primary liver damage (e.g., hepatitis, cirrhosis) or biliary obstruction (e.g., stone, tumor structure), is accompanied by increased amounts of coproporphyrin I in the urine and decreased amounts in the feces. This results from diversion of excretion of this substance from the bile to the urine.

2. Excessive exposure to certain toxic agents, including barbiturates, lead, and alcohol, may result in increased urinary excretion of coproporphyrin III.

This occurs also in cirrhosis in alcoholic subjects, in contrast to the increase in Type I in "nonalcoholic" cirrhosis.

3. Increased amounts of coproporphyrin I are excreted in the urine in hemolytic disorders and other conditions accompanied by stimulation of erythropoiesis. Excretion of this substance, formed as a by-product of heme synthesis (p. 204), approximately parallels that of urobilinogen.

4. The basis for the increase in urinary coproporphyrin III in poliomyelitis is not known. Nor is the cause of this phenomenon clear in such conditions as aplastic anemia and Hodgkin's disease. It may conceivably be due in some instances to impaired liver function, and in certain cases of aplastic anemia to unsuspected exposure to toxic chemical agents.

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CHAPTER VI

Biochemical Aspects of Diet

In general terms, an adequate diet is one which permits normal growth, maintenance, and reproduction. More specifically, its minimum requirements for the adult are fulfilled (1) when the organism is in a state of equilibrium with regard to (*a*) calories, (*b*) nitrogen, and (*c*) inorganic elements; (2) when there is an adequate supply and utilization of vitamins and other factors (e.g., trace minerals, essential fatty acids) necessary for maintenance of special functions; (3) when reproduction is normal. In the immature organism, growth and development must proceed normally.

The functions served by the essential components of the diet and the quantities required for optimum nutrition are considered in detail elsewhere in discussing the role of each of these factors in biochemical processes, and need not be repeated here. However, certain general aspects of the subject of diet merit independent consideration.

It is necessary to appreciate the difficulties involved in arriving at satisfactory standards of diet that can be applied to large groups of peoples of different dietary habits and economic status. The bulk of present information regarding fundamental aspects of nutrition has emerged from studies in experimental animals. Despite the existence of important species differences in many respects, fortunately it has been found possible to utilize much of this information directly in practical problems of nutrition in man. Nevertheless, there are still large areas of uncertainty in important fields. For example, even in experimental animals, there is no assurance that an adequate diet, as described above, is an optimum diet. There are, in fact, indications that such is not the case. Moreover, because of the integration of various phases of intermediary metabolism and the functional interdependence of several of the factors involved, construction of a satisfactory diet must include consideration of quantitative interrelationships between certain of these principles or their precursors in the foods. For example, the protein requirement is influenced by the level of carbohydrate in the diet and also by the caloric intake; the requirement for various B vitamins is influenced by the carbohydrate, caloric, or protein intakes. These and other difficulties, some recognized and some unknown, make it necessary to provide for a rather wide margin of safety in order to insure satisfactory nutrition. The valuable recommendations of the Food and Nutrition Board of the National Research Council, indicated in Table 7, constitute an important contribution to this subject.

The food intake is influenced by geographical and economic factors as well as by personal idiosyncrasies. The diet of the Eskimo is limited by his environment largely to meat and fish. Large numbers of Orientals are forced to subsist mainly

Table 7. Food and Nutrition Board, National Research Council,

(Designed for the Maintenance of Good Nutrition of Healthy Persons in the U.S.A.; Temperate

	AGE *Years*	WEIGHT *kg. (lb.)*	HEIGHT *cm. (in.)*	CALORIES	PROTEIN *gm.*
Men	25	65 (143)	170 (67)	3200[2]	65
	45	65 (143)	170 (67)	2900	65
	65	65 (143)	170 (67)	2600	65
Women	25	55 (121)	157 (62)	2300[2]	55
	45	55 (121)	157 (62)	2100	55
	65	55 (121)	157 (62)	1800	55
	Pregnant (3rd trimester)			Add 400	80
	Lactating (850 ml. daily)			Add 1000	100
Infants[3]	0–1/12[4] (No specific recommendations)				
	1/12–3/12	6 (13)	60 (24)	kg. x 120	kg. x 3.5[3]
	4/12–9/12	9 (20)	70 (28)	kg. x 110	kg. x 3.5[3]
	10/12–1	10 (22)	75 (30)	kg. x 100	kg. x 3.5[3]
Children	1–3	12 (27)	87 (34)	1200	40
	4–6	18 (40)	109 (43)	1600	50
	7–9	27 (59)	129 (51)	2000	60
Boys	10–12	35 (78)	144 (57)	2500	70
	13–15	49 (108)	163 (64)	3200	85
	16–20	63 (139)	175 (69)	3800	100
Girls	10–12	36 (79)	144 (57)	2300	70
	13–15	49 (108)	160 (63)	2500	80
	16–20	54 (120)	162 (64)	2400	75

[1] In planning practical dietaries, the recommended allowances can be attained with a variety of common foods which will also provide other nutrient requirements less well known; the allowance levels are considered to cover individual variations among normal persons as they live in the United States subjected to ordinary environmental stresses. Other nutrients discussed in the text include: fat, p. 217; water, p. 295; sodium chloride, p. 274; phosphorus, p. 224; iodine, p. 264; vitamin K, p. 468.

[2] These calorie recommendations apply to the degree of activity for the average active man and woman. For the urban "white-collar" worker they are probably excessive. In any case, the calorie allowance must be adjusted to the actual needs of the individual as required to achieve and maintain his desirable weight.

on rice, with variable supplements of other vegetable and fish. As a general principle, any type of rigid dietary restriction, voluntary or forced, carries with it the danger of deficiency in some essential factor. The greater the variety of foods, the more remote this danger becomes. Of less practical importance, perhaps, in normal subjects, is the manner of preparation of foods, i.e., seasoning, flavoring, cooking, except in that improper procedures may destroy or remove certain vitamins or impair the quality of the food proteins. These matters may assume importance, however, in maintaining adequate nutrition in disease, increased palatability of the food causing stimulation of appetite.

Recommended Daily Dietary Allowances,[1] *Revised 1953*

Allowances are Considered to Apply to Persons Normally Vigorous and Living in Climate)

CALCIUM *gm.*	IRON *mg.*	VITAMIN A *I. U.*	THIAMINE *mg.*	RIBOFLAVIN *mg.*	NIACIN *mg.*	ASCORBIC ACID *mg.*	VITAMIN D *I. U.*
0.8	12	5000	1.6	1.6	16	75	
0.8	12	5000	1.5	1.6	15	75	
0.8	12	5000	1.3	1.6	13	75	
0.8	12	5000	1.2	1.4	12	70	
0.8	12	5000	1.1	1.4	11	70	
0.8	12	5000	1.0	1.4	10	70	
1.5	15	6000	1.5	2.0	15	100	400
2.0	15	8000	1.5	2.5	15	150	400
0.6	6	1500	0.3	0.4	3	30	400
0.8	6	1500	0.4	0.7	4	30	400
1.0	6	1500	0.5	0.9	5	30	400
1.0	7	2000	0.6	1.0	6	35	400
1.0	8	2500	0.8	1.2	8	50	400
1.0	10	3500	1.0	1.5	10	60	400
1.2	12	4500	1.3	1.8	13	75	400
1.4	15	5000	1.6	2.1	16	90	400
1.4	15	5000	1.9	2.5	19	100	400
1.2	12	4500	1.2	1.8	12	75	400
1.3	15	5000	1.3	2.0	13	80	400
1.3	15	5000	1.2	1.9	12	80	400

[3] The recommendations for infants pertain to nutrients derived primarily from cow's milk. If the milk from which the protein is derived is human milk or has been treated to render it more digestible, the allowance may be in the range of 2–3 gm. per kg. of body weight. There should be no question that human milk is a desirable source of nutrients for infants even though it may not provide the recommended levels for certain nutrients. (See discussion in text, pp. 218–220.)

[4] During the first month of life desirable allowances for many nutrients are dependent upon maturation of excretory and endocrine functions. Therefore no specific recommendations are given.

Dietary considerations of inorganic elements are discussed elsewhere (p. 274). We shall review here certain pertinent points regarding the caloric, carbohydrate, fat, and protein intakes insofar as they bear on biochemical aspects of the diet. More detailed discussions of vitamin and other dietary requirements are available in standard works on nutrition.

Caloric Content of Diet. The daily caloric requirements under various physiological conditions are considered in detail elsewhere (p. 371). These are, of course, contributed by the three classes of organic foodstuffs, carbohydrate, fat, and protein. From the standpoint of adequate nutrition, of these the protein requirement is the most rigid, the accepted recommendation being not less than 1 gram per kilogram of body weight daily. This amount (e.g., 70 gm. = 280 Cal.) would contribute about 10 per cent of the energy requirement of a moderately active 70-kg. man. It is somewhat higher in pregnant and lactating women and in children (Table 7). The relative proportions of carbohydrate and fat contributing the remainder could vary widely, but it is recommended that carbohydrate contribute at least 50 per cent and fat at least 20 per cent of the total calories. Increased energy demands incident to increased activity may be met by either of the two latter foodstuffs. However, ketone bodies may accumulate in excess if carbohydrate provides much less than half of the total calories on a high-fat diet.

If the caloric intake is insufficient to meet current demands, the body tissues will supply the deficit. If the protein intake is adequate and caloric restriction of only moderate degree, this will be derived from depot fat after the small glycogen reserves have been depleted. However, with progressive reduction in caloric intake, a deleterious influence is exerted on protein metabolism, as reflected in nitrogen balance, this effect becoming more evident after the fat reserves have been utilized. With severe, prolonged restriction, virtually all tissues lose weight, but not equally, fat, liver, and muscle suffering considerably, whereas brain, plasma proteins, and bones are maintained for relatively long periods.

The organism adapts itself to caloric restriction to a certain extent by an associated decrease in the rate of oxidations. The BMR may decrease by about 50 per cent after prolonged semi-starvation, owing in part to the above phenomenon, but mainly to the decreased mass of metabolizing tissue.

The requirement for certain vitamins is influenced by the caloric intake. This is particularly true of the B vitamins that are concerned in oxidative mechanisms, the requirement for which diminishes as the caloric content of the diet is reduced. In fact, frank vitamin deficiency manifestations are not often seen in total starvation, but may develop during subsequent feeding if the intake of vitamins is not increased in proportion to the caloric intake.

Dietary Carbohydrates. Inasmuch as all biologically significant carbohydrates can be synthesized in the body, their main function in the diet is to provide a readily utilizable source of energy for the maintenance of cell functions. Under usual conditions, carbohydrate should provide 50 per cent or more of the calories of the diet, i.e., about 5 to 7 grams per kilogram of body weight in the average moderately active adult.

Undue restriction of dietary carbohydrate influences both fat and protein metabolism adversely, even if the caloric intake is adequate. Fat mobilization from the depots and utilization are exaggerated (p. 91), ketogenesis is increased (p. 94), and ketosis may develop. The effect on protein metabolism is apparently of a specific nature, not shared by other substances (e.g., fat, alcohol), and not related to its calorigenic action. This is referred to as the "protein-sparing action" of carbohydrate. During a total fast, an adult may lose about 70 grams of protein daily. This may be reduced to about 35 grams by a daily intake of 100 grams of glucose, which represents approximately its maximum protein sparing effect under these circumstances. It has been estimated that a maximum effect in this connection is accomplished by 5 grams of glucose per 100 Calories produced.

Celluloses, indigestible plant polysaccharides, contribute bulk to the intestinal contents and, therefore, in normal amounts promote intestinal motility. When present in excess, they may be irritating to the intestinal mucous membrane, producing diarrhea or a spastic type of constipation. All other dietary carbohydrates are readily digested and/or absorbed. Ingestion of excessive amounts, especially in infants, may occasionally produce intestinal disturbances due to irritation induced by products of bacterial fermentation. There is some evidence in experimental animals (rat) that continued ingestion of supertolerance amounts of galactose may result in the formation of cataract. A similar phenomenon has been suggested as possibly occurring in man, especially in children, in the presence of hepatic functional impairment, in which condition the conversion of galactose to glucose (or glycogen) may be impaired, with consequent elevation of the blood galactose concentration. It should be recalled in this connection, however, that the incidence of cataract is relatively high also in uncontrolled diabetes, a phenomenon presumably related in this case to maintenance of a persistently elevated blood glucose concentration.

Dietary Lipid. A wide variety of lipids is provided in a balanced diet. Inasmuch as, under normal circumstances, all components of biologically significant lipids, with the exception of certain "essential" fatty acids (p. 82), can be synthesized in the body from nonlipid precursors, the main function of dietary lipids, like that of carbohydrates, is to provide energy, largely through their constituent fatty acids. The dietary lipids serve other, indirect functions in serving as a carrier of certain vitamins and provitamins (e.g., A, D, E, K), which, because of their solubility in fats, occur in nature mainly in association with these substances. There is also some indication that they may exert a relatively minor protein-sparing effect, apart from their caloric contribution. Neutral fats, comprising the largest fraction of food lipids, are quantitatively the most important of these substances. Under usual conditions, fat provides 20 to 35 per cent of the calories of the diet, i.e., 1 to 2 grams per kilogram of body weight in the average moderately active adult.

The nutritional significance of the polyunsaturated "essential" fatty acids is discussed elsewhere (p. 82). Suffice it here to state that they are provided in ample amounts by a balanced diet that contains an adequate quantity of naturally occurring lipids (linoleic mainly in plants, arachidonic animal sources).

Inasmuch as most of the common food fats contain mainly the same few fatty acids, viz., oleic, palmitic, and stearic, although in different proportions, there is little significant difference in their digestibility or biological value, whether of plant or of animal origin. When ingested in amounts that are not excessive, all of those commonly used in cooking or as foods are well digested and absorbed, most of them to the extent of about 95 per cent, but somewhat less in the case of beef and mutton fat. Of more academic than practical importance is the fact that triglycerides with unusually high proportions of longer chain saturated fatty acids, resulting in high melting points (above 38° C.), may remain solid at body temperature, and, therefore, are less readily digested and/or absorbed.

An excessively high fat intake inhibits gastric secretion and motility, producing anorexia and gastric discomfort. Intestinal irritation and diarrhea may result from excessive amounts of fatty acids in the intestine. Although of little practical importance, it is of interest that cottonseed oil contains a pigment, gossypol, which has antoxidant and antitryptic activity, diminishes appetite, and interferes with protein digestion.

Dietary Protein. The large amount of information available on nutritive aspects of food proteins has been obtained mainly from studies in the albino rat and to a limited extent in the dog (especially plasma proteins and hemoglobin). The relatively few observations that have been made on human subjects support the view that, with occasional exceptions, the results of studies in these experimental animals may be generally applied to man.

Food proteins perform a vitally important function different from that served by dietary carbohydrates and fats, the main purpose of which is to provide an adequate amount of energy. The proteins of the diet are the ultimate source of amino groups, and of the carbon skeletons of the essential amino acids required for synthesis of body proteins, which comprise the bulk of the metabolic machinery (e.g., enzymes) and of other structural and functional components of the tissues and body fluids. Taken in excess, they may also be utilized for the production of energy; they may be converted to carbohydrate or to fat. Inasmuch as all body proteins are continually undergoing degradation (p. 127) and there is an obligatory loss of nitrogen in the urine, at least this amount of nitrogen must be supplied in the diet if protein anabolism is to keep pace with its catabolism, even in the adult, nongrowing state. The diet must also provide certain "essential" amino acids that cannot be synthesized in the body.

Inasmuch as growth involves formation of tissue proteins at a rate exceeding that of their degradation (i.e., positive N Balance), the dietary protein is much more critically concerned in growth and tissue repair than are carbohydrates and lipids. This influence may be demonstrated readily in the growing animal, whose weight curve reflects the adequacy (quantity and quality) of the protein intake. There is evidence also that the latter determines to a significant extent the ultimate adult size of the individual, over and above genetic influences. Adult heights and weights of peoples in various geographic areas apparently vary directly with the amount and quality of protein in their diets. For example, members of certain African tribes subsisting largely on vegetables are small in stature, whereas neighboring tribes of similar origin, subsisting mainly on meat and milk, are tall. Similar correlations have been obtained in studies of genetically comparable groups of individuals in this and other countries.

Emphasis was placed originally on the quantitative protein requirements almost exclusively. With the development of more satisfactory analytical methods for amino acids, and the accumulation of information on "essential" amino acids, it became apparent that the "quality" of dietary proteins was just as important as their quantity in contributing to adequate protein nutrition.

Nitrogen equilibrium can be maintained, i.e., protein requirements can be satisfied, just as satisfactorily by intravenous as by oral administration of adequate mixtures of amino acids. The same is true of plasma proteins. Moreover, apart from the influence of certain unknown factors present in natural animal proteins (e.g., animal protein factor), intravenous injection of such mixtures can completely replace oral protein feeding, at least for periods of several days. Properly fortified protein (e.g., casein), hydrolysates and various mixtures of pure amino acids are commercially available for this purpose, and have aided considerably in maintaining satisfactory nutrition in patients in whom adequate oral feeding is impossible or undesirable, e.g., after operations on the gastrointestinal tract.

QUALITY OF FOOD PROTEINS (BIOLOGICAL VALUE). Food proteins vary considerably in the efficiency of their utilization for synthesis of body proteins (e.g., tissues, hemoglobin, plasma proteins). Any one of several procedures may be employed for evaluation of the "quality" of a protein: (1) measurement of its influence on the weight increase of weaning animals ("protein efficiency ratio" $= \frac{\text{weight increase (grams)}}{\text{grams protein consumed}}$); (2) determination of its "biological value" in terms of the percentage of absorbed nitrogen retained by the organism; (3) measurement of its influence on the rate of regain of body weight or of liver protein by previously depleted animals; (4) measurement of its influence on the rate of restoration of plasma proteins or of hemoglobin in animals previously depleted of these specific proteins (e.g., by plasmapheresis). In the case of hemoglobin, a conjugated protein, consideration must be given to other factors, including iron (p. 259) and requirements for porphyrin synthesis (p. 205) and erythropoiesis (viz., vitamin B_{12}).

Evaluated on the basis of these criteria, animal proteins generally are of higher "quality" than those of vegetable origin. Whole egg and milk (especially lactalbumin) proteins rank highest in this respect, meats and glandular tissues somewhat lower. Gelatin, commonly used in desserts, although a protein of animal origin (not occurring naturally as such), is a wholly inadequate protein, lacking tryptophan, valine, and tyrosine, and being low in cystine. It is prepared from collagen (cartilage, bone, tendon, skin) by boiling in water.

Certain factors are known to influence the biological value of proteins: (1) the amounts and relative proportions of their constituent amino acids; (2) the nutritional availability (rates of liberation and absorption) of their constituent amino acids under conditions of digestion in the gastrointestinal tract; (3) the latter factor (2) may be influenced, adversely or beneficially, by various methods of processing (commercial) or preparation (cooking) of foodstuffs.

AMINO ACID COMPOSITION. The most important single factor, but not the only one, that influences the nutritional "quality" of a protein is its amino acid composition. The significance of the "essential" amino acids in this connection is discussed elsewhere (p. 130). These must be provided in the diet if nitrogen equilibrium is to be maintained. Inasmuch as they must all be presented to the organism virtually simultaneously for purposes of protein synthesis, a "complete" food protein must contain all of them. However, the biological value of a protein is apparently related to the proportionality relationships among its constituent essential amino acids. The following daily intake values (in grams) have been recommended tentatively: isoleucine 1.4, leucine 2.2, lysine 1.6, methionine 2.2, phenylalanine 2.2, threonine 1.0, tryptophan 0.5, valine 1.6. These are about double the minimal requirements and should provide an adequate excess, from which each cell may select the proper mixture for its specific protein synthesis requirements.

Despite the fact that the organism can manufacture the "nonessential" amino acids, when a mixture of the "essential" amino acids is the only source of dietary nitrogen, growth is not as satisfactory as when the same amount of nitrogen is supplied in the form of intact complete proteins or their hydrolysates, under otherwise comparable conditions (i.e., carbohydrate and fat contents). This is apparently due, in part at least, to the fact that the presence in the diet of a "nonessential" amino acid, e.g., cystine, may reduce the requirement for an "essential" amino acid, in this case methionine. It has been suggested that when the former are not provided, the necessity for their synthesis may constitute an excessive burden on the metabolic activities of the cell. This is particularly true under conditions of rapid growth (childhood, pregnancy, convalescence from wasting diseases).

Availability of Amino Acids of Foods. Different proteins with similar amino acid compositions may not liberate a given amino acid at the same rate during the course of digestion, and the rates at which different amino acids are liberated may vary widely. An apparent determining factor in this connection is the nature of the amino acid linkage within the protein molecule. Digestibility of various proteins and the rates of release of amino acids may be affected by the manner of preparation of foods (e.g., heating), in some cases adversely, in others beneficially. On this basis, wide differences in the time of absorption of individual amino acids from the intestinal lumen may diminish the nutritional usefulness of a protein of satisfactory chemical constitution because of the importance of the time element in the utilization of amino acids for protein synthesis (p. 221).

Supplementary Relationships; Time Factor. If a relatively low-quality protein food, such as white bread (low in lysine), is supplemented by a small amount of lysine, raising its content of this amino acid from 0.29 to 0.67 per cent, its biological value is more than doubled. The utilization of an "incomplete" protein for protein synthesis is limited by the amino acid least available ("law of the minimum"). Similarly, mixtures of food proteins, each of which is "incomplete" in different respects, may provide a satisfactory amino acid balance for optimum protein nutrition.

Analysis of various types of staple protein foodstuffs, reveals that their most significant deficiencies are in lysine, methionine, threonine, and tryptophan. For example, wheat, which is an important dietary protein, is deficient in lysine; this is true of cereal grains generally. Rice is, in addition, poor in threonine, and corn in tryptophan. Leguminous (peas, beans) and root vegetable proteins are generally deficient in methionine, the former also in tryptophan. The combination of wheat (low in lysine, but adequate in methionine) and potatoes or peas (adequate in lysine, but low in methionine) raises the biological value of each of these relatively low-quality proteins considerably. Similar enhancement of the utilization of relatively low-quality (vegetable) proteins is accomplished by the addition of high-quality (animal) proteins.

A protein molecule can be synthesized only if all of its constituent amino acids are available simultaneously in proper amounts. If an interval of longer than one hour is allowed to elapse between ingestion of an incomplete amino acid mixture and administration of the missing essential amino acids, these amino acid constituents are not utilized for protein synthesis. Under these circumstances, weight loss is much the same as on an equicaloric diet containing no amino acids. The same applies, of course, to mixtures of dietary proteins that supplement one another's deficiencies; they must be ingested simultaneously in order to be maximally utilized. There is evidence, too, that even high-quality dietary proteins are utilized more efficiently when distributed evenly throughout the three daily meals than if concentrated in one or two meals. The time of administration of carbohydrate in relation to protein is also of importance in this connection (p. 131).

QUANTITY OF PROTEIN. If the intake of protein is reduced gradually, urinary nitrogen excretion diminishes correspondingly and the organism may remain in nitrogen equilibrium until a critical intake level (about ¼–⅓ gram per kilogram of body weight) is reached, below which the nitrogen balance becomes negative. However, continuation of such low-protein intakes for prolonged periods may endanger health and is certainly undesirable. Animals maintained on such diets are more susceptible to subsequent protein depletion than are those receiving high-protein diets.

It has been found that protein nutrition is maintained satisfactorily in adults by a daily intake of ⅔ gram per kilogram of body weight. Larger amounts are required during pregnancy (growth of fetus, placenta, uterus, breasts), lactation (production of milk proteins), childhood (body growth), and convalescence from wasting diseases (tissue repair). In order to provide for variations in nutritional quality and for fluctuations in requirement incident to contingencies of everyday existence, the Food and Nutrition Board of the National Research Council has recommended the following daily allowances (Table 7, p. 214) per kilogram of body weight; normal adults, 1 gram; during the latter half of pregnancy, 1.45 grams; during lactation, 1.8 grams; infants, 3.5 grams; early childhood, 2.2 to 3.3 grams; late childhood through adolescence, 1.4 to 2 grams.

The daily requirement is, of course, influenced by the quality of the food protein. For example, whereas nitrogen equilibrium can be maintained on 0.6 gram (per kilogram of body weight) of milk protein daily, over 1 gram (per kilogram of body weight) of white flour protein is required for this purpose. The dietary protein recommendations indicated presuppose that the diet is adequate in other respects and that high-quality proteins (whole egg, milk, meats, liver) comprise at least half of the total protein intake in the case of normal adults, half to two-thirds during pregnancy and lactation, and two-thirds to three-quarters during childhood and adolescence.

Although there is evidence that an extremely high protein intake may produce kidney damage in the rat, there is no substantial evidence that this is true in man within feasible limits of dietary protein content.

CONSEQUENCES OF PROTEIN DEFICIENCY. If the protein intake does not meet the immediate requirements, protein anabolism cannot be maintained at the required rate. In the child, growth is retarded; in the adult, weight is lost, wound healing is delayed. Hemoglobin synthesis is impaired, with consequent anemia. If the deficiency is marked, excessive amounts of fat may accumulate in the liver (p. 108), a consequence of deficiency in methionine, and degenerative changes and fibrosis (cirrhosis) may occur. Prolonged deficiency may result in inadequate synthesis of plasma proteins. If this progresses to the point of significant decrease in the plasma albumin concentration, edema may develop (p. 155), and also increased susceptibility to shock. Resistance to infections may be diminished as a result of impaired capacity for forming γ-globulins (antibodies) (p. 159). In severe protein restriction, certain hormones, protein in nature, such as those of the anterior pituitary, may not be synthesized in adequate amounts, and endocrine abnormalities may appear, e.g., amenorrhea (gonadotrophin deficiency). Since enzymes are proteins and must be synthesized in the body, the enzyme content of certain tissues (e.g., liver) and secretions (e.g., pepsin in gastric juice) falls in advanced deficiency states. This may result in disturbances of function of the organs affected.

BENEFICIAL AND DELETERIOUS FACTORS IN FOODS. Apart from their protein content, certain animal foodstuffs (e.g., milk, liver) contain an unidentified factor (or factors) which enhances protein synthesis and growth. This has been termed "animal protein factor" (APF), and perhaps will eventually be found to belong in the category of vitamins; vitamin B_{12}, before it was separated from this "factor," was included under this designation. This APF may contribute to the biological superiority of animal over vegetable proteins.

Certain foodstuffs, in their natural state, may contain nutritionally deleterious components. For example, soybeans contain a principle with antitryptic activity which depresses appetite and interferes with protein digestion, an effect similar to that of gossypol in cottonseed oil. This substance, presumably protein in nature, is inactivated by heat, which explains, in part at least, the fact that the nutritional value of soybean is increased considerably by proper cooking technics.

Toxic materials may be present in certain foods, as consumed, as a result of either natural occurrence or processing. These include such factors as lead, arsenic, selenium, and copper, and a substance present in wheat flour bleached with agene (NCl_3) (now prohibited), which causes serious nervous system manifestations (e.g., "canine hysteria"). Protection is afforded against many of these recognized hazards by appropriate legislation. A factor in fava beans may induce a hemolytic disorder, favism. Spoiled sweet clover hay contains dicoumarin (p.

470), which, when ingested by cattle, produces a hemorrhagic condition characterized by prothrombin deficiency.

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CHAPTER VII

Calcium and Inorganic Phosphate Metabolism

Although over 99 per cent of the body calcium and 80 to 85 per cent of its phosphorus is in the bones, important functions of these elements are exerted in directions other than preservation of skeletal structure. Ca ions (*a*) decrease neuromuscular excitability, (*b*) decrease capillary and cell membrane permeability, and are necessary for (*c*) normal muscle contraction, (*d*) normal transmission of nerve impulses, and (*e*) blood coagulation. Ca ions also activate certain enzymes, an action which is undoubtedly involved in several of the functions mentioned above.

Phosphorus is involved in various important phases of organic metabolism. It is necessary merely to recall here the fundamental role of high-energy phosphate bonds in the storage, liberation, and transfer of energy (e.g., ATP, phosphocreatine) (p. 11), the importance of hexose- and triose-phosphates in the intermediary metabolism of carbohydrate (p. 5), and the metabolic significance of such P-containing substances as phospholipids (p. 83), nucleic acids (p. 192), and nucleotides (DPN, TPN, etc.) (p. 196). We shall be concerned here with the role of phosphate in inorganic metabolism.

The metabolism of calcium is intimately related to that of phosphate in many important respects, and it is therefore convenient to discuss the two substances together in order to avoid repetition.

ABSORPTION

Calcium and phosphate are absorbed in the small intestine, more readily in the upper than in the lower portions. In the course of digestion of nucleoproteins and phosphoproteins, phosphate may be split off and absorbed as such. If ester forms are present, they may undergo hydrolysis (enzymes of pancreatic and intestinal juice) prior to absorption. It has been suggested that because of this fact a considerable fraction of the dietary phosphate is absorbed later than the major portion of the calcium. This may permit better absorption of both elements, especially in regions of relatively low acidity.

Several factors influence the degree of absorption of calcium:

(1) Other factors being equal, absorption of calcium is roughly proportional to its *concentration in the intestine*. The same appears to be true of phosphate.

(2) As in the case of iron, but not to the same extent, the amount of calcium absorbed varies somewhat with the *requirement of the organism for this element*.

(3) *Intestinal pH.* Calcium salts, particularly phosphates and carbonates, are quite soluble in acid solutions and are relatively insoluble in alkaline solutions. Consequently, factors which increase intestinal acidity favor absorption of Ca, and vice versa. Under conditions of normal gastric acidity, Ca salts of weak organic acids are converted to the soluble chloride and, if retained in the stomach for a sufficient period, even the less soluble basic phosphate may go into solution. The acidity in the duodenum, normally pH 2.3 to 7.0, is of considerable importance, determining whether more of the Ca is in the form of the more soluble acid phosphate or less soluble basic phosphate. Calcium chloride and acid phosphate are probably absorbed from the duodenum before the gastric juice acidity is neutralized. Subsequently, absorption of Ca may be favored by the presnce of organic acids (e.g., lactic, citric, amino acids, fatty acids).

(4) *Other Substances in the Food Mixture.* An excess of Mg apparently diminishes absorption of Ca, especially if the latter is not present in adequate amounts. Considerable interest has centered in the Ca : P ratio, the optimum ratio for absorption of both elements being about 1 : 1 (1 : 2 to 2 : 1). An excessively high ratio is accompanied by decreased absorption of phosphate (rachitogenic diet, p. 243). It is stated that a low ratio does not result in decreased Ca absorption. This effect is apparently related to the influence on solubility of the products (calcium phosphates) formed under these conditions. Such substances as iron, beryllium, aluminum and strontium, which form insoluble phosphates, likewise interfere with absorption of phosphate, and can induce rickets (p. 243).

Phytic acid (inositol hexaphosphate), which occurs in cereal grains, forms insoluble salts (phytin) with Ca and Mg (insoluble above pH 3 to 4), with consequent impairment of absorption of these elements. This phenomenon is regarded as the basis of the rachitogenic effect of such cereals as oatmeal. Hydroxyacids such as lactic, citric and tartaric shift the precipitation point of Ca phytate toward a higher pH (see also action of vitamin D, below, and p. 465).

(5) *Vitamin D.* Vitamin D promotes absorption of Ca from the distal ileum, where absorption is otherwise relatively poor, but not from the upper ileum, where Ca is absorbed more readily. Intestinal absorption of phosphate is apparently increased somewhat. This may be a result of increased Ca absorption, maintaining an optimum local Ca : P ratio.

Vitamin D apparently counteracts the effect of phytic acid in binding the Ca^{++} ions and thus diminishing Ca absorption. In adequate amounts and with a high Ca intake, it suppresses the rachitogenic and anticalcifying effect of phytic acid. The mechanism of its action in this connection is not known.

Vitamin D lowers the pH in the distal ileum, cecum and colon. This may facilitate Ca absorption or, on the other hand, may be a result, in part at least, of increased absorption, decreasing the base of the intestinal contents.

BLOOD CALCIUM AND PHOSPHATE

The inorganic phosphate of blood plasma ranges from 3 to 4.5 mg./100 ml. (1.7–2.5 mEq/l.) in adults and from 4.5 to 6.5 mg. (2.5–3.6 mEq/l.) in children. It is somewhat higher in summer than in winter (solar ultraviolet irradiation).

It decreases during periods of increased carbohydrate utilization (administration of glucose or insulin), due presumably to increased utilization for phosphorylation of carbohydrate metabolites (hexose- and triose-phosphates). A slight increase follows ingestion of large amounts of calcium and a considerable drop follows parenteral administration of magnesium.

There is no calcium in erythrocytes. The calcium content of plasma (usually determined in serum) is 8.5 to 11.5 mg./100 ml. (usually 9–11 mg., or 4.5–5.5 mEq/l.). During infancy and early childhood (to twelve years) the average values approach the upper limit of this range, falling with advancing years.

Calcium exists in the plasma in two physiologically distinct fractions, designated (*a*) diffusible or ionized and (*b*) nondiffusible or nonionized. The diffusible fraction (50 to 60 per cent of total) is capable of passing through an artificial semipermeable membrane (cellophane, collodion) and, presumably, through the living capillary wall; the nondiffusible fraction (40–50% of total) cannot, due to its combination with plasma proteins. Although the two fractions may vary independently of each other under certain abnormal circumstances (e.g., hypoproteinemia, in which the nondiffusible Ca alone may be decreased; hypoparathyroidism, in which the diffusible fraction alone may be decreased), they seem to be in a state of rather unstable equilibrium, which is probably controlled to a certain extent by the parathyroid hormone. This agent exerts its influence primarily and almost entirely on the diffusible (ionized) fraction, which probably is the only physiologically active portion of the serum calcium.

The ionized fraction of the serum calcium, normally about 4.5 to 5.0 mg./100 cc., can be measured directly by a biological procedure (i.e., isolated frog heart), which is not suitable for routine clinical purposes. Inasmuch as the nondiffusible (nonionized) fraction is related quantitatively to the serum protein concentration, the calcium ion concentration can be estimated from values for total calcium and total protein (Fig. 24).

Maintenance of the plasma Ca concentration within a narrow range is of vital concern to the organism inasmuch as this ion has a profound influence on certain fundamental processes affecting cell function (e.g., membrane permeability, neuromuscular excitability). A few of the factors concerned in the preservation of the normal level of Ca in the plasma deserve special consideration.

Parathyroid Hormone. In the fasting state (i.e., no absorption from the intestine), the plasma Ca concentration is maintained primarily by its mobilization from the bones through the action of the parathyroid hormone. This is the most important factor in the regulation of the concentration of Ca ions in the body fluids. Its physiological actions, which are exerted in the metabolism of both calcium and phosphorus, may be illustrated by outlining the consequences of (1) removal of the parathyroid glands, and (2) injection of extracts of these glands (parathyroid hormone).

EFFECTS OF PARATHYROIDECTOMY. The following phenomena follow removal of all parathyroid tissue: (1) decreased output of phosphate in urine; (2) rise in serum phosphate concentration; (3) decreased urinary calcium output; (4) de-

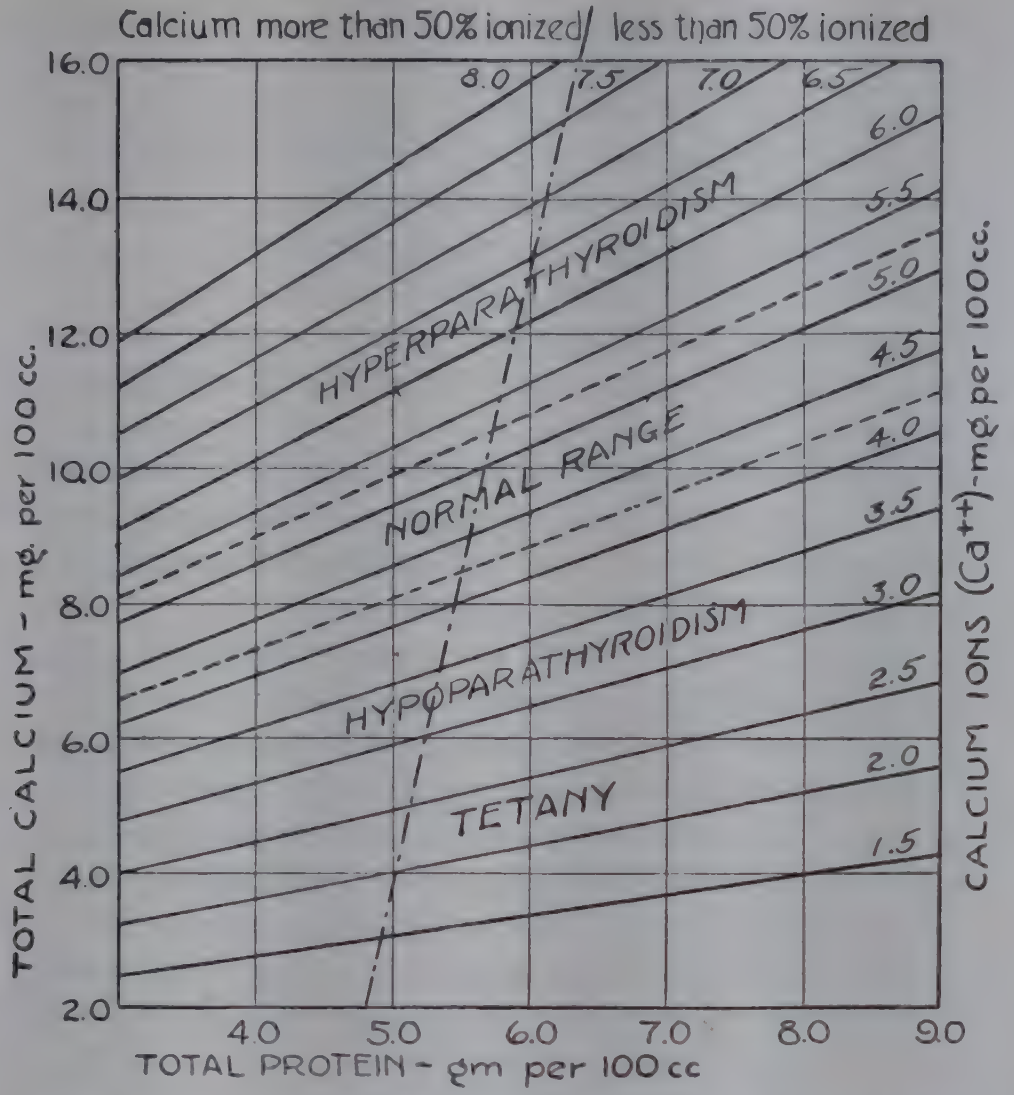

Fig. 24. Chart for calculation of $[\text{Ca}^{++}]$ from total protein and total calcium of serum or plasma. (McLean, F. C. and Hastings, A. B.: Am. J. Med. Sc., *189*:601, 1935.)

crease in serum calcium, largely if not entirely in the diffusible (ionized) fraction.

Effects of Parathyroid Hormone Injection. The chief consequences of administration of this hormone are: (1) increased urinary phosphate excretion; (2) decrease in serum phosphate; (3) increased urinary calcium excretion; (4) increase in serum calcium concentration; (5) increased serum alkaline phosphatase activity.

Mechanism of Action. Present evidence suggests that the parathyroid hormone exerts a dual effect. (1) It decreases renal tubular reabsorption of inorganic phosphate from the glomerular filtrate; excessive amounts of hormone therefore cause excessive loss of phosphate in the urine and a fall in its concentration in the plasma. (2) It accelerates mobilization of skeletal materials, including calcium, with consequent increase in calcium excretion in the urine, increased calcium concentration in the plasma, and skeletal demineralization (p. 233). The increase in serum alkaline phosphatase activity in hyperparathyroidism is a reflection of increased osteoblastic activity, i.e., stimulation of osteoid formation (p. 250).

CONTROL OF PARATHYROID SECRETORY ACTIVITY. There is no evidence to indicate nervous or hormonal control of parathyroid function. Secretory activity is apparently regulated by the plasma calcium concentration, a decrease in which stimulates and an increase depresses parathyroid hormone secretion. Inasmuch as the effect of this hormone is to raise the level of calcium in the blood, this is an example of a very efficient autoregulatory mechanism, in which the concentration of a constituent of the blood regulates itself.

Vitamin D. As indicated elsewhere (p. 225), the action of vitamin D in maintaining the normal plasma Ca concentration depends chiefly on its effect in enhancing absorption of Ca from the intestine. In the presence of vitamin D deficiency, the plasma PO_4 usually falls sooner than the Ca concentration. This is due, in part at least, to stimulation of parathyroid activity by the decreasing plasma Ca concentration, which tends to maintain the normal Ca level and, simultaneously, to lower the plasma phosphate.

Plasma Proteins. Calcium exists in the plasma in higher concentration than would be possible in the absence of protein. As indicated above (p. 226), about half of the plasma (nondiffusible fraction) is bound to plasma proteins, principally albumin, decrease in which may be accompanied by a decrease in the serum Ca concentration. The calcium-binding potentialities of the plasma proteins may not be "saturated" under normal conditions, so that frequently no significant fall in serum Ca occurs at slight or moderate levels of hypoproteinemia. Such variations, since they are confined to the nondiffusible Ca fraction, are not accompanied by manifestations of altered Ca metabolism or neuromuscular excitability (e.g., tetany), and hypocalcemia of this type (i.e., due to hypoproteinemia) does not cause stimulation of parathyroid function.

Plasma Phosphate. There is a roughly reciprocal relationship between the concentrations of Ca and PO_4 ions in solutions in vitro. In the body fluids, this relationship is exhibited to a limited extent and practically only in one direction; i.e., rather marked increase in serum PO_4 may cause a fall in serum Ca concentration. This may be demonstrated experimentally by intravenous injection of Na_2HPO_4, and is encountered clinically in advanced renal failure with high serum PO_4 concentrations (p. 243).

Acid-Base Equilibrium. The well-known occurrence of tetany in alkalosis, in the presence of normal total calcium concentrations, suggests that the plasma pH may influence the degree of ionization of the serum calcium. There has been no direct evidence of such an effect. However, the belief has been prevalent for many years that there is a quantitative relationship between the concentrations of hydrogen, bicarbonate, and phosphate ions, as expressed in the following formula:

$$\frac{[Ca^{++}]\,[HCO_3^-]\,[HPO_4^=]}{[H^+]} = k \text{ (constant)}$$

According to this concept, the concentration of calcium ions decreases as the bicarbonate and phosphate ion concentrations increase, and as the hydrogen ion concentration decreases.

Miscellaneous. Ingestion of adequate amounts of soluble calcium salts in the postabsorptive state results in elevation of serum calcium, which reaches a maximum in two to three hours and returns to the previous level in about four hours. After intravenous injection, the peak is reached in a few minutes, with a subsequent fall to normal, usually within one to two hours, depending upon the quantity given. Following intramuscular injection (calcium glucogalactogluconate) a maximum level is reached in about one hour, with a subsequent fall over a period of three to four hours. Intravenous or intramuscular injection of magnesium salts may cause a fall in serum calcium, at times to tetanic levels.

DEPOSITION AND MOBILIZATION OF BONE MINERALS

There is no unanimity of opinion regarding either the essential chemical nature or the mechanism of deposition of the mineral components of bone. The most important of the ionic constituents are Ca (about 26% of the dry weight), PO_4 (about 12%), CO_3 (about 3.5%), and smaller amounts of Mg, Na, Na, F, and citrate. On the basis of chemical analysis and x-ray diffraction studies, it would appear that the basic bone mineral has a crystal structure similar to that of the apatite group of phosphate minerals, probably a hydroxyapatite. As the crystal grows it is exposed to other elements and molecular groupings which cannot be incorporated in the lattice, the most important of which are carbonate, citrate, Mg, Na, F, Sr. These, with additional Ca and PO_4, are deposited on the surface of the hydroxyapatite crystal, stopping its growth. In bone, a new surface is started by the development of an independent crystal. In the case of dental enamel, a new surface is started by covering over the previous one.

The surface deposits are not part of the chemical structure of the fundamental bone mineral and are therefore more readily removed than the latter without actual dissolution of bone substance. Studies with radioactive elements indicate that these materials may be divided into two categories; (1) elements or molecular groupings which can displace or replace normal constituents of bone both on the surface and in the interior of the crystals; metabolic turnover of these components is relatively slow; (2) elements or molecular groupings which can enter a surface reaction with bone crystals, but which cannot be incorporated in the crystal lattice; metabolic turnover of these components is relatively rapid. It is believed that not more than 12 per cent of the skeletal calcium can be mobilized without actual dissolution of bone substance, including the matrix. This readily exchangeable calcium is in the form of salts (carbonate, citrate, phosphate) on the surface of the bone crystals, not in the actual lattice.

Mineralization of Bone. Bone formation involves two distinct fundamental processes: (1) construction of an organic matrix and (2) deposition of bone salts in this matrix. In areas of developing bone, reticular cells of the invading marrow turn into osteoblasts, which appear to be responsible for laying down the intercellular organic matrix (osteoid), which is largely protein in nature (p. 246). Ascorbic acid and vitamin A are essential for the normal formation of this organic matrix. The osteoblasts subsequently turn into osteocytes, the mature bone cells. When bone undergoes destruction, the osteocytes and osteoblasts form osteoclasts, which are actively involved in the breakdown of bone; these can in turn be transformed to osteoblasts or reticular cells.

Proper mineralization requires provision of Ca and PO_4 ions, at the site, in their proportions in the mineral matter of bone. Mineralization will not occur if there is a deficit in one of these ions even though the other is present in adequate amounts. They are provided in the concentrations present in the interstitial fluid, in which they of course exist in solution. Understanding of the process of mineralization of bone must involve an explanation of the mechanism underlying the precipitation of calcium phosphate at this site. This is not entirely clear.

Alkaline phosphatase is present invariably in areas of active mineralization, suggesting that it is involved in this process. It is present in particularly high concentration in osteoblasts and, in skeletal disorders, its concentration in the blood plasma reflects the degree of osteoblastic activity (p. 251). It was originally believed that this enzyme hydrolyzes organic esters of phosphoric acid in the developing bone, producing a local increase in PO_4 ion concentration with consequent precipitation of calcium (local supersaturation). However, there is no evidence that such esters are present in these situations in amounts adequate to produce this effect. It has been suggested that phosphatase favors mineralization: (*a*) by increasing the concentration of PO_4 ion; (*b*) by increasing the concentration of Ca^{++} ion by removing a complexing agent (phosphoric esters); (*c*) by preventing absorption of ester phosphate, thereby eliminating a process which inhibits growth of the mineral crystals.

Recently, attention has been directed toward the possibility that glycolysis may be involved in this process. Pre-osseous cartilage contains glycogen, phosphorylase, ATP and lactic acid, important components of the glycolytic cycle. Cartilage cells preparatory to mineralization accumulate large amounts of glycogen, which disappears abruptly just before or during the course of deposition of bone salts. Whether glycogenolysis provides a source of phosphoric ester substrate for phosphatase, or whether its main purpose is the provision of energy for some phase of the mechanism of bone formation is not clear.

Vitamin D is necessary for normal skeletal mineralization. The mode of its action in this connection is not known; it may perhaps provide optimum conditions of Ca and PO_4 concentration in the bone (p. 465). It is also essential for the normal growth of bones, exerting a specific effect at the zone of provisional calcification in long bones and at corresponding sites in membranous bones (p. 466). Defects in this mechanism result in rickets.

Mobilization of Calcium. In common with other tissues, bone undergoes continual metabolic turnover, its various components undergoing degradation, mobilization and replacement. This is true also of its mineral constituents. Studies with radioactive Ca have shown, as mentioned above, that this element occurs in two types of deposit (i.e., bone salt and surface deposit) which differ in their lability. Less than 12 per cent of the skeletal calcium is readily mobilizable and replaceable, probably without dissolution of the osteoid matrix; the remainder is much more stable and probably cannot be mobilized without simultaneous breakdown of osteoid tissue. Moreover, the mineral constituents of the trabecular portions of bone are more labile than those of the cortex.

The parathyroid hormone is the most important physiological factor involved in the mobilization of Ca from the skeleton. It apparently acts directly on the bones, causing its dissolution and resorption, the liberated calcium entering the blood stream. This is the major factor in the maintenance of the concentration of Ca ions in the plasma and tissue fluids (p. 226). Under normal conditions this process is balanced by resynthesis of osteoid components and mineralization of the newly formed matrix.

MISCELLANEOUS FACTORS

Estrogens and androgens exert an influence in calcium metabolism which varies widely in different species. Marked hypercalcemia occurs in birds during and just before ovulation, and may also be induced by administration of estrogen. This increase is largely in the nondiffusible fraction. Although this phenomenon does not occur in mammals, in rats estrogens produce hyperossification of the proximal epiphyseal zone of the tibia and thickening of the trabeculae, filling the upper fifth of the diaphyseal marrow cavities. This may be prevented by administration of androgens. It is questionable whether these observations are applicable to man. However, after the menopause, a type of osteoporosis often develops, with negative Ca balance, which may be benefited by administration of estrogen and/or androgen.

Osteoporosis and negative Ca balance may occur in subjects with hyperthyroidism or hyperfunction of the adrenal cortex. This may be due to the protein catabolic action of excessive amounts of thyroid hormone and the adrenal 11-oxysteroids, affecting primarily the osteoid matrix and secondarily, therefore, skeletal mineralization.

Decreased alkalinity of the body fluids (acidosis), due to any cause, may result in increased urinary excretion of calcium. The excess is presumably removed from the bones because of the increased solubility of the labile calcium deposits in a more acid medium.

EXCRETION

Normal adults on an adequate calcium intake are in a state of calcium equilibrium. Ca is excreted in the urine, bile, and digestive secretions. The bulk of that in the feces is food Ca which has escaped absorption. This represents a variable but considerable fraction of the intake, since even under optimal conditions dietary Ca (e.g., in milk) is not more than 50 per cent absorbed. A small amount of the fecal Ca may be unabsorbed Ca of the digestive fluids.

At low and moderate levels of Ca intake (0.1–0.5 gm. daily), about 30 to 35 per cent is excreted in the urine, while at high levels of intake (1.0 gm.) about 10 to 25 per cent is so eliminated. However, there is considerable deviation from these values, due probably to variable dietary, metabolic and gastrointestinal factors. For example, conditions which interfere with absorption of Ca from the intestine will increase the proportion excreted in the feces at the expense of urinary excretion. These include vitamin D deficiency, high phytin intake, achlorhydria or ingestion of alkalis, diarrhea, etc. The "renal threshold" for Ca excretion lies between 6.5 and 8.0 mg. per 100 cc. plasma, little being eliminated at lower serum Ca concentrations.

Inorganic phosphate is excreted in the urine and feces, the relative proportions varying under different conditions, e.g., those which influence absorption from the intestine (q.v.). The source of urinary inorganic phosphate is chiefly that of the blood plasma, although it may be contributed to by hydrolysis of phosphoric acid esters by phosphatase activity in the kidneys. On a balanced diet, urine phosphate constitutes about 60 per cent of the total excretion. As the Ca intake is decreased, the proportion eliminated in the urine increases (increased intestinal absorption), being about 75 per cent on a low Ca, moderately high PO_4 intake. The "renal threshold" for phosphate excretion is about 2 mg./100 cc. of plasma, excretion falling to very low levels at lower concentrations. Reabsorption of phosphate from the glomerular filtrate by the renal tubular epithelium is apparently inhibited by the parathyroid hormone. Administration of this agent (or clinical hyperparathyroidism) causes increased urinary excretion of phosphate.

CALCIUM AND PHOSPHORUS REQUIREMENT

The adult daily requirement for phosphorus is 1 to 1.5 gm., or about 1.5 times the calcium intake. Diets adequate in other respects provide an adequate amount of phosphorus because of its wide distribution in common foods (phosphoproteins and phospholipids, inorganic phosphate), expecially milk and milk products, wheat, meats, and fish.

The minimal daily adult requirement for calcium is 0.45 gm., but the recommended allowance is 1.0 gm. (10–15 mg./kg. body weight), increasing to 1.5 gm. or more during pregnancy and lactation. Growing children require about 45 mg./kg. body weight. Even under optimal conditions, not more than 35 to 50 per cent of dietary calcium is absorbed. Milk (1.0 gm. per quart) and dairy products constitute the most important dietary source of this element, cereals, meats and most vegetables being low in calcium. Even those which contain relatively large amounts (viz., certain vegetable greens, and shell-fish) are usually not eaten in sufficient quantities to be of practical value in this regard. The average American diet is probably usually deficient in calcium, which may be supplied satisfactorily in the form of inorganic or organic salts (e.g., phosphate, lactate, chloride, gluconate).

CALCIUM CONTENT OF OTHER BODY FLUIDS

It is probable that the cerebrospinal fluid and normal tissue fluids contain the diffusible fraction of serum calcium, namely, 4.5 to 5 mg. per 100 cc. In the presence of protein in tissue fluids their calcium content is increased by an amount proportional to the quantity of protein present, the added calcium being nondiffusible in nature and the diffusible fraction remaining within the limits above mentioned.

Under conditions of abnormal serum calcium concentration and partition, as in hypo- and hyperparathyroidism, the cerebrospinal fluid calcium concentration does not vary directly, quantitatively, with the quantity of either total or diffusible serum calcium.

ABNORMAL SERUM CALCIUM

Deviation from the normal state of calcium in the blood serum may be manifested in one of two ways, namely, by an alteration in the total serum calcium concentration or by an alteration in the distribution or partition of calcium in the blood and tissue fluids.

Hypercalcemia

Hyperparathyroidism. One of the most striking results of the injection of parathyroid hormone is an increase in the level of total serum calcium, the degree of rise being dependent upon the dosage and the frequency of its administration. This hypercalcemia occurs even though calcium is withheld from the diet, the increased quantity of circulating calcium being derived from the bones. The sequence of metabolic events has been indicated elsewhere (p. 227). The clinical picture of hyperparathyroidism induced in experimental animals by prolonged administration of excessive amounts of parathyroid hormone is virtually identical with that occurring spontaneously in man as a result of hypersecretion by neoplastic or hyperplastic parathyroid glands (generalized osteitis fibrosa cystica; von Recklinghausen's disease of bone).

The characteristic, indeed almost pathognomonic metabolic manifestations are: (*a*) hypercalcemia, (*b*) hypophosphatemia, (*c*) increased urinary excretion of calcium, and (*d*) increased serum alkaline phosphatase activity. It is the combination of several or all of these, rather than the presence of any one, that is of diagnostic significance.

Serum calcium values ranging from 12 to 29.5 mg. per 100 cc. have been observed in these cases, but values above 20 mg. are rare. Occasionally the serum calcium may be within normal limits, but repeated determinations usually reveal hypercalcemia at some time during the course of the disease. Hypercalcemia is probably invariably present during periods of progression of the skeletal lesions and in the presence of active symptoms. It is probable that there may be periods of temporary latency of the condition, as in the case of hyperthyroidism. There is also experimental basis for the possibility that the serum calcium may return to normal or even subnormal levels following a period of hypercalcemia, with exhaustion of the mobilizable calcium reserves in the bones, particularly if the calcium intake is inadequate. However, this must occur rarely, if at all, clinically. Demonstration of abnormally high calcium and phosphate excretion in the urine (pp. 240, 245) is of great diagnostic importance in all questionable cases.

In uncomplicated cases, the increase in serum calcium is accompanied and, in fact, preceded by a decrease in serum phosphate 1.0 to 2.5 mg./100 cc., the latter phenomenon being of considerable diagnostic significance (p. 244). Occasionally, in cases of primary hyperparathyroidism complicated by renal failure, a not uncommon complication (nephrocalcinosis, nephrolithiasis, pyelonephritis), the serum calcium concentration may fall to normal levels coincidently with increase in serum phosphate concentration (pp. 242, 623). Under such circumstances the condition may be confused with "renal rickets" (p. 623), but as a rule diagnosis is not difficult.

The serum alkaline phosphatase activity is generally increased (p. 250), usually ranging from 6.5 to 25 Bodansky units, and occasionally as high as 60 units. This is a reflection of osteoblastic activity, i.e., stimulation of bone repair.

As indicated above, renal failure may supervene as a result of complicating nephrolithiasis or renal calcification, often with superimposed infection (pyelonephritis). The clinical picture then includes metabolic manifestations of renal functional impairment, e.g., nitrogen retention, acidosis, hyperphosphatemia.

Rarely, the condition may be fulminating (acute hyperparathyroidism), resembling acute experimental parathyroid intoxication, with vomiting, diarrhea, shock, and renal failure.

Parathyroid hyperplasia, and presumably increased secretory activity ("secondary hyperparathyroidism"), occur rather consistently in conditions not originating in these glands. These include:

1. Chronic glomerulonephritis, renal hypogenesis, and other forms of chronic renal disease associated with "renal rickets" (p. 242).
2. True rickets and osteomalacia (vitamin D deficiency).
3. Various types of chronic steatorrhea (sprue, celiac disease).
4. Pregnancy and lactation.
5. Certain cases of senile osteoporosis, multiple myeloma, pituitary basophilism, acromegaly, osteogenesis imperfecta, marble-bone disease, and chronic hypertrophic arthritis.

Except perhaps in the latter group, the parathyroid enlargement and hypersecretion occur in response to a decrease in the plasma calcium concentration, the normal stimulus to parathyroid activity (p. 228). In some cases, this results from decreased absorption from the intestine (vitamin D deficiency, sprue, celiac disease), in others from hyperphosphatemia (renal failure). In pregnancy, it is a consequence of the increased requirement for calcium during the period of growth of the fetal skeleton. During lactation, the serum calcium tends to decrease as a result of the excretion of calcium in the milk.

There should be no confusion between primary hyperparathyroidism and these conditions of "secondary hyperparathyroidism," With the exception of the last-named group of miscellaneous skeletal diseases, which probably do not belong in this category, hypercalcemia does not occur in these disorders. The enlargement and hyperactivity of the parathyroid glands constitute a mechanism designed to maintain a normal plasma calcium level in the face of a tendency toward hypocalcemia. If the blood calcium concentration should rise above normal, parathyroid activity would be depressed automatically, with prompt decrease in the serum calcium. This fact, together with differences in serum phosphate and phosphatase activity, usually aids materially in distinguishing between true, i.e., primary hyperparathyroidism and "secondary hyperparathyroidism."

Hypervitaminosis (Vitamin D). Hypercalcemia may result from the administration of excessive quantities of vitamin D. There appears to be a wide range of individual susceptibility to the introduction of this factor and the coincident administration of large quantities of calcium salts increases the tendency toward the production of hypercalcemia. Cases have been reported in which skeletal demineralization, with hypercalcemia and metastatic calcification in various situations, especially the kidneys and the blood vessels, followed daily administration of as little as 50,000 units daily for a few weeks. In others, toxic manifestations appeared only after administration of 500,000 units daily for six to twelve months. Pre-existing renal disease predisposes to the development of this condition.

The administration of therapeutic doses of dihydrotachysterol (A.T. 10), a substance chemically related to vitamin D, results in an increase in serum calcium, The physiologic effect of this substance appears to be more closely analogous to that of the parathyroid hormone than does that of vitamin D. It is used commonly in the treatment of hypoparathyroidism.

Multiple Myeloma. Serum calcium values ranging from normal to 20.2 mg. per 100 cc. have been observed in patients with multiple myeloma. Hypercalcemia has been reported in about 50 per cent of the cases in which mention was made of the serum calcium concentration, the values being above 12 mg. in 20 to 45 per cent. Some have attributed this phenomenon to the existence of a state of hyperparathyroidism. However, this hypothesis is contradicted by the usual finding in multiple myeloma of a normal or elevated serum phosphorus concentration (renal functional impairment) and usually normal serum phosphatase activity. The increase in serum calcium may be dependent in some cases upon the frequently increased serum protein concentration. However, the serum calcium is normal in many cases with marked hyperproteinemia, and may be elevated in the presence of normal total protein concentrations. It seems probable that actual destruction of bone by the progressing skeletal lesion, with liberation of bone minerals into the blood stream, is the main underlying cause of hypercalcemia in multiple myeloma. This is contributed to undoubtedly by the presence of renal functional impairment, a common feature of this disease.

Neoplastic Disease of Bone. The serum calcium concentration is usually within normal limits in the great majority of cases of primary and metastatic neoplasms of bone. However, values as high as 22 mg. per 100 cc. have been reported, particularly in cases of extensive metastatic involvement of the skeleton. In such cases, values for serum phosphorus and serum phosphatase activity are usually within normal limits, except in the case of osteogenic sarcoma, in which the serum phosphatase activity may be increased.

In recent years, large doses of androgen and estrogen have been employed rather extensively in the treatment of inoperable mammary carcinoma with skeletal metastases. Severe hypercalcemia may develop in such cases, usually with rapid progression of the osteolytic lesions, presumably the source of the added calcium. The serum calcium may rise rapidly to extremely high levels, i.e., over 20 mg./100 cc. The hypercalcemia is accompanied by nausea, vomiting, and dehydration, and may, in severe cases, result in precipitation of calcium in the renal tubules with consequent progressive renal damage and renal functional insufficiency. The serum inorganic phosphate is usually elevated simultaneously.

Acute Bone Atrophy. Hypercalcemia, with increased urinary excretion of calcium and phosphorus, may occur as a result of rapid demineralization of bones, especially in the presence of simultaneously impaired renal function. This has been observed particularly in children, during periods of immobilization of the extremities due to fracture or paralysis (poliomyelitis). In contrast to hyperparathyroidism, the serum phosphate is either normal or elevated. Serum alkaline phosphatase activity is normal or low.

Kidney Disease. As is indicated elsewhere (p. 623), the serum calcium may be low in patients with various types of kidney disease as a result of hypoproteinemia (p. 238) or hyperphosphatemia, i.e., renal failure (p. 239). However, occasional instances have been reported of hypercalcemia in subjects with renal functional impairment, usually of relatively mild degree. This is due apparently to inadequate excretion of calcium, and is therefore aggravated by an unusually high calcium intake. There have been several reports of the occurrence of hypercalcemia (without hypercalciuria) in patients with peptic ulcer who ingested excessive amounts of alkali and milk. Alkalosis may contribute to the renal functional impairment in such cases. Metastatic calcification may occur, and this, as well as the hypercalcemia, may disappear after discontinuing the high milk and alkali intake.

Miscellaneous. Hypercalcemia is encountered occasionally in patients with sarcoidosis, values above 14 mg./100 cc. having been reported. The combination of hypercalcemia, increased serum alkaline phosphatase activity, hypercalciuria, urinary calculi, and skeletal lesions may superficially be suggestive of hyperparathyroidism, but this differential diagnosis is seldom difficult on the basis of other criteria.

Slightly elevated values have been reported in occasional cases of polycythemia vera, acromegaly, and Cushing's syndrome, and in patients with a respiratory type of acidosis (primary increase in CO_2 tension), e.g., severe emphysema and pulmonary fibrosis. The cause and significance of hypercalcemia in such cases are not clear.

Hypocalcemia

Hypoparathyroidism. Hypocalcemia is one of the most constant features of diminished parathyroid function. Clinically, this condition occurs in two forms, (*a*) postoperative, following thyroidectomy or parathyroidectomy, and (*b*) idiopathic, a condition analogous to spontaneous hypothyroidism. A third rare variety may follow hemorrhage or inflammation in the deep cervical tissues or in the glands themselves. Postoperative tetany may result from accidental removal of the parathyroid glands during thyroidectomy or from excision of too much parathyroid tissue in parathyroidectomy for hyperparathyroidism. Occurring after thyroidectomy, it is usually due to temporary suppression of parathyroid function as a result of trauma, edema, or hemorrhage, or interference with the blood supply.

The characteristic metabolic features of hypoparathyroidism are: (*a*) hypocalcemia, (*b*) normal or increased serum P concentration, (*c*) decreased urinary excretion of Ca and P, with positive Ca and P balances, (*d*) normal serum phosphatase activity, (*e*) normal acid-base equilibrium, and (*f*) no significant skeletal abnormality; perhaps increased bone density.

One of the most important manifestations is the diminished serum Ca concentration. This is usually between 7 and 8 mg. per 100 cc. in latent, and between 4 and 6 mg. per 100 cc. in manifest tetany. However, this close parallelism between the severity of symptoms and the degree of hypocalcemia is not constant. Latent tetany may be present at normal or at low (6 to 7 mg. %) serum Ca levels and manifest tetany may occur at concentrations as high as 8 mg. per 100 cc. Such discrepancies indicate that other factors must participate in the pathogenesis of neuromuscular hyperirritability in this condition. Perhaps the most important of these is the serum P concentration. This is at times normal, but is usually increased (5 to 10 mg. %). Even when it is not elevated, the serum Ca/P ratio is decreased. It appears likely that this absolute or relative increase in serum P plays a significant role in determining the precipitation of clinical symptoms of tetany (below). From the standpoint of differential diagnosis, particularly from tetany due to vitamin D deficiency, the elevated or normal serum P concentration, positive Ca and P balances, normal phosphatase activity, and absence of skeletal abnormality are of paramount importance.

In the absence of parathyroid hormone the organism is unable (*a*) to excrete normal amounts of inorganic phosphate in the urine and (*b*) to mobilize calcium from the bones as actively as normally. In subjects with no functioning parathyroid tissue, the serum calcium may fall to 5 mg. per 100 cc., or even lower, and the serum phosphate may rise to 12 mg. per 100 cc.; calcium may disappear from the urine when the serum calcium falls below 7 to 8 mg. The consensus is that the hypocalcemia is accompanied by a decrease in the ionized fraction. The nondiffusible fraction may or may not be decreased.

The relation of these chemical changes to the state of neuromuscular hyperirritability which is a dominant characteristic of the parathyroprivic organism is not entirely clear in certain details. The prevailing view is that the increased irritability is due predominantly to a decreased concentration of ionized calcium in the circulating fluids, altering the balance between the monovalent cations, Na and K, and the divalent cations, Ca and Mg. However, there are many observations that indicate that this is not the only factor involved, although it is perhaps the most important one. An increase in inorganic phosphorus accentuates both the hypocalcemia and the tetany, although, as in rickets, hypocalcemic tetany can occur without a significant increase in serum phosphate. As a rule, however, an increase in serum phosphate concentration aggravates the tetanic manifestations at any given level of hypocalcemia, probably by decreasing the calcium ion concentration (p. 228).

If parathyroidectomized rats are fed aluminum acetate, the serum phosphorus is low, the serum calcium normal, and there is no tetany. If the serum phosphorus rises as a result of either excessive tissue catabolism or diet, the serum calcium falls and tetany develops. Agents such as hypertonic saline, hypertonic glucose, and acids may relieve parathyroid tetany by producing a phosphate diuresis, lowering the serum phosphate concentration.

Pseudohypoparathyroidism. Cases have been observed presenting the metabolic and clinical picture of hypoparathyroidism, but in which the fundamental disturbance appeared to be diminished end-organ responsiveness rather than merely lack of hormone. In contrast to true hypoparathyroidism, large doses of parathyroid hormone exert little or no effect on the serum calcium and phosphorus excretion in these cases. They are also somewhat resistant to dihydrotachysterol.

Vitamin D Deficiency (Rickets and Osteomalacia). Deficiency in vitamin D (p. 466) results characteristically in rickets in young children and osteomalacia in older children and adults. In the majority of cases the serum calcium is within normal limits, the serum phosphate being characteristically decreased and the serum alkaline phosphatase activity increased. In some instances, however, especially when the calcium intake is low, hypocalcemia occurs (4–7.5 mg. per 100 cc.), with manifestations of tetany (infantile tetany; spasmophilia; osteomalacic tetany).

Although the primary effect of vitamin D deficiency is apparently to diminish absorption of calcium from the intestine, hypocalcemia is usually not an early manifestation. This is due presumably to the fact that the tendency toward lowering of the serum calcium concentration stimulates the parathyroids (enlargement and hypersecretion), with consequent increased mobilization of skeletal calcium and lowering of the serum phosphate concentration. Hypocalcemia appears when this compensatory mechanism becomes inadequate, e.g., when the readily mobilizable calcium of the bones has been exhausted.

Steatorrhea (Sprue, Celiac Disease). The outstanding feature of these disorders is defective absorption of fat from the intestine and diarrhea. There is probably also deficient absorption of vitamin D. These abnormalities lead to inadequate calcium absorption, rickets, dwarfism, osteomalacia, hypocalcemia and tetany. The serum phosphate is often low and excessive amounts of calcium and phosphate are lost in the feces.

Hunger Osteopathy. This condition appears to be due to a diet deficient in total calories, calcium, phosphorus and vitamin D, and resembles a slowly progressive osteomalacia.

Nephrosis. Serum calcium values ranging from 5.7 to 9.1 mg. per 100 cc. have been observed in patients with nephrosis in the absence of renal failure. This diminution in serum calcium is due entirely to a decrease in the nondiffusible fraction which occurs as a result of a marked diminution in the concentration of serum protein, which is one of the most characteristic features of this condition. In no case is there any significant alteration in the amount of diffusible calcium and increased neuromuscular excitability is not observed.

Hypocalcemia in nephrotic children has been found to be accompanied by a pronounced decrease in excretion of calcium in the urine and an increase in the feces, with generalized rarefaction of diaphyseal portions of the bones.

Mild grades of hypocalcemia have been reported in other conditions accompanied by hypoproteinemia, e.g., kala-azar, malignancy, prolonged obstructive jaundice, and severe protein malnutrition.

Nephritis. Hypocalcemia is occasionally observed in chronic glomerulonephritis with nitrogen retention. It occurs usually in the later stages of this condition and is associated with and perhaps dependent upon the increase in serum phosphate which occurs in these cases. In advanced nephritis with uremia, inorganic phosphorus figures of 12 to 20 mg. per 100 cc. may be associated with serum calcium values of 4 to 6 mg. per 100 cc. Tetany may occur as a result of such marked lowering of the serum calcium concentration. As was indicated above, the concentration of calcium in the serum varies roughly inversely with that of phosphorus. A similar situation may develop in chronic renal failure due to any cause, e.g., pyelonephritis, urinary obstruction, renal hypogenesis, polycystic disease, etc. The hypocalcemia, particularly in chronic glomerulonephritis, may be contributed to by a coexisting hypoproteinemia, as indicated above (i.e., nephrotic syndrome).

Maternal Tetany. Certain physiologic changes in calcium and phosphorus metabolism in pregnancy are mentioned elsewhere (p. 403). The requirement for these elements and for vitamin D is increased and the occurrence of parathyroid hyperplasia indicates the increased functional demands upon these structures. Under normal conditions the serum calcium tends to fall, but not to subnormal levels, during the late months of pregnancy and during lactation, but it may become subnormal occasionally, with mild manifestations of tetany. The etiology of this condition is not clear, but it appears to be dependent in some cases upon deficiency in calcium and vitamin D and in others upon parathyroid deficiency operating alone or in conjunction with other factors. The interesting condition of "tetany of the newborn" appears to be related to the existence of tetany in the mother. It is due perhaps to inability of the maternal parathyroid glands to compensate fully for the fetus and to the fact that the fetal parathyroids are not fully functioning at birth in such cases.

Magnesium, Phosphate, Oxalate and Citrate Tetany. Intravenous or intramuscular injection of magnesium salts may cause a fall in serum calcium, at times to tetanic levels.

A fall in serum calcium, with signs of tetany, may follow intravenous injection of neutral, alkaline or slightly acid sodium phosphate. That tetany does not occur after injection of acid sodium phosphate, even though the serum calcium falls, is due probably to increase in calcium ions incident to the accompanying acidosis. The hypocalcemia of chronic renal failure probably results chiefly from phosphate retention (p. 242).

Parenteral administration of soluble oxalates and of citrates produces tetany, the former by the production of hypocalcemia through the formation of insoluble calcium oxalate, the latter by formation of a poorly ionized calcium citrate compound, without hypocalcemia.

Acute Pancreatitis. Hypocalcemia has been reported to occur in the majority of cases of acute hemorrhagic pancreatitis (up to 70%), the values usually ranging

Table 8. Conditions Commonly Associated with Alteration in Serium Calcium Concentration and Partition

CONDITION	TOTAL CA, MG. PER 100 CC.	DIFFUSIBLE CA, MG. PER 100 CC.	PHOSPHATE, MG. PER 100 CC.	ALKALINE PHOSPHATASE UNITS	PROTEIN, GM. PER 100 CC.	pH
Tetany:						
Parathyroid	Decrease	Decrease	Increase	Normal	Normal	Normal
Maternal	Decrease	Decrease	Normal or increase	Normal or increase	Normal or decrease	Normal
Infantile	Decrease	Decrease	Decrease or normal	Increase	Normal	Normal
Osteomalacic	Decrease	Decrease	Decrease or normal	Increase	Normal	Normal
Gastric	Normal	Normal	Normal	Normal	Normal	Increase
Hyperventilation	Normal	Normal	Normal	Normal	Normal	Increase
Bicarbonate	Normal	Normal	Normal	Normal	Normal	Increase
Sprue	Decrease	Decrease	Decrease or normal	Normal or increase	Normal or decrease	Normal
Celiac disease	Decrease	Decrease	Decrease or normal	Normal or increase	Normal or decrease	Normal
Hyperparathyroidism	Increase	Increase	Decrease	Increase	Normal	Normal
Nephrosis	Decrease	Normal	Normal	Normal	Decrease	Normal
Renal failure	Decrease	Decrease	Increase	Normal	Decrease	Decrease

between 7 and 8 mg./100 cc., but occasionally falling below 7 mg. This phenomenon has been attributed to the sudden removal of large amounts of calcium from the extracellular fluids, including the blood plasma, as a result of its fixation as insoluble calcium soaps by fatty acids in areas of fat necrosis. The validity of this explanation has not been established.

Alkalosis. It is probable that in alkalosis there is a diminution in the proportion of ionized calcium which is responsible for the symptom complex, tetany, so characteristic of alkalotic states. The serum calcium in these conditions is usually within normal limits, the disturbance probably taking place in accordance with the ionization formula referred to previously (p. 228). This condition occurs clinically following the administration of excessive quantities of alkali (bicarbonate tetany), in states of hyperventilation (hyperpneic tetany), and in association with pyloric and acute upper intestinal obstruction or excessive vomiting from any cause (gastric tetany), the level of total serum calcium being normal in each instance.

ABNORMAL URINE CALCIUM

Increased Urinary Calcium

Hyperparathyroidism. The injection of parathyroid extract is followed by a gradual increase in the excretion of calcium, this increase taking place entirely in the urinary calcium, the fecal excretion being unaffected. Although in most cases the increased elimination is dependent upon a rise in the level of serum calcium, it may occur in the absence of any such elevation. Similar observations have been made in clinical states of hyperparathyroidism (osteitis fibrosa diffusa). Whereas under normal conditions 70 to 90 per cent of the excreted calcium is eliminated in the feces and 10 to 30 per cent in the urine, in hyperparathyroidism these proportions are reversed, 70 to 90 per cent being eliminated in the urine and only 10 to 30 per cent in the feces. Patients suffering with this condition cannot be maintained in calcium equilibrium upon a daily intake sufficient for normal individuals (0.45 gm. calcium daily). This negative balance is best demonstrated when the intake of calcium and phosphorus is maintained at a low level for test periods of at least three days (calcium 0.11 gm. and phosphorus 0.4 gm. daily). This test procedure is of diagnostic value in early or mild cases of hyperparathyroidism, when the abnormalities in serum calcium and phosphorus are not striking or characteristic.

Hyperthyroidism. Increased thyroid secretion is associated with a marked accentuation of calcium excretion, the urinary calcium being increased somewhat out of proportion to the fecal calcium and averaging about 56 per cent of the total output. This appears to be due to a direct stimulating catabolic effect on the calcium deposits in the bones similar to the general action of thyroid extract upon other body tissues.

Acidosis. Acidosis, whether due to the ingestion of mineral acids or acid-forming substances or associated with disease states, is accompanied by an increase in calcium excretion, the excess calcium, as in the case of hyperthyroidism and hyperparathyroidism, being in all probability abstracted from the bones. This increased excretion of calcium is due almost entirely to an increase in urinary calcium.

Hypervitaminosis D. The administration of extremely large quantities of vitamin D or of smaller doses of dihydrotachysterol (A.T. 10) may result in increased excretion of calcium in the urine similar to that observed in hyperparathyroidism. In the case of vitamin D, this may represent a toxic rather than a physiologic effect.

Idiopathic Hypercalciuria. In the majority of cases of nephrolithiasis (calcium oxalate or phosphate calculi) no metabolic abnormality can be demonstrated other than excretion of excessive amounts of calcium. The mechanism underlying this type of hypercalciuria is not known.

Decreased Urinary Calcium

Hypoparathyroidism. In hypoparathyroidism, hypocalcemia is associated with a diminution in the urinary excretion of calcium. Recent studies appear to indicate that there is a threshold for urinary calcium excretion since the elimination of calcium in the urine decreases abruptly as soon as the serum calcium falls below 6.5 to 8.5 mg. per 100 cc.

Vitamin D Deficiency. Urine calcium is decreased in vitamin D deficiency, as occurs in rickets and osteomalacia and perhaps also in certain cases of steatorrhea (sprue, celiac disease). This is perhaps due largely to inadequate absorption of calcium from the intestine.

Hypothyroidism. In myxedema, the excretion of calcium has been found to be approximately 40 per cent below the normal average, the diminution in urinary calcium being somewhat greater proportionately than that in fecal calcium.

Ingestion of Bases. It has been found that the administration of base-forming diets causes a decrease in urinary calcium. It is probable that these effects are due largely to decreased absorption from the intestine rather than to any direct influence upon the intermediary metabolism of calcium.

Miscellaneous. An unusually low excretion of calcium, as compared with normal subjects, has been reported in patients with calcinosis universalis maintained on a low calcium and phosphorus intake. A marked diminution in the output of calcium in the urine is a rather constant finding in chronic nephritis. This is due in part to impaired renal excretion of calcium and in part to inadequate absorption of calcium resulting from excretion into the intestine of large amounts of phosphorus which would have been excreted in the urine under conditions of normal renal function. This, together with the accompanying acidosis, may contribute to development of the condition known as "renal dwarfism" or "renal rickets," an occasional accompaniment of chronic renal failure in children. Secondary hyperparathyroidism plays a part in the pathogenesis of the skeletal changes in this condition (p. 234). Urinary calcium is also decreased, and may be virtually absent, in children with chronic nephrosis.

ABNORMAL FECAL CALCIUM

An increase in the quantity of calcium eliminated in the feces may be observed in rickets and osteomalacia (vitamin D deficiency), in sprue and in celiac disease. In the last two conditions this increase is probably due to defective calcium absorption resulting from diarrhea and inadequate absorption of vitamin D. In rickets and osteomalacia, however, although defective absorption may be of importance, it is believed by many that the fundamental defect is in the intermediary metabolism of calcium and phosphorus, whereby normal ossification cannot occur and an increased quantity of calcium is eliminated into the lower bowel. Conversely, calcium retention may be produced by the administration of excessive quantities of vitamin D with consequent diminution in the amount of calcium eliminated in the feces. Fecal excretion of calcium may be excessive in children with chronic nephrosis.

HYPERPHOSPHATEMIA

Hypervitaminosis (Vitamin D). The serum phosphate concentration may be increased by large doses of vitamin D in the form of cod liver oil or viosterol. Similar results may be obtained by ultraviolet irradiation.

Hypoparathyroidism. Diminished parathyroid function is associated with a slight rise in the serum phosphate concentration which is more or less proportional to the diminution in serum calcium.

Renal Failure. Increase in the concentration of phosphate in the serum of patients with chronic glomerulonephritis is an indication of renal functional insufficiency. Phosphate retention in nephritis appears to contribute to the acidosis which occurs in the later stages of that condition, high values for serum phosphate being almost invariably accompanied by a decrease in the alkali reserve. However, acidosis may exist in the absence of demonstrable phosphate retention. Hyperphosphatemia in nephritis has approximately the same clinical significance as creatinine retention, values above 8 mg. per 100 cc. in adults being of serious prognostic import. Values as high as 40 mg. per 100 cc. have been reported. Similar alterations in the serum phosphate concentration may occur in renal insufficiency associated with nephrosclerosis, multiple myeloma and destructive kidney lesions such as congenital polycystic kidney, tuberculosis, malignancy, pyonephrosis, pyelonephritis and hydronephrosis. An increase in serum inorganic phosphorus concentration has also been observed in acute high intestinal obstruction. This may possibly be dependent upon or associated with the state of renal functional insufficiency which occurs in that condition. A similar change has been observed after injection of histamine.

Healing Fractures. During the period of healing of fractures in adults the serum phosphate concentration is often slightly increased, values ranging from 5 to 7 mg. per 100 cc. being frequently observed.

HYPOPHOSPHATEMIA

Rickets. In rachitic children, serum phosphate values of 1 to 2 mg. per 100 cc. are commonly observed. This condition is usually due fundamentally to vitamin D deficiency, the mode of operation of which is discussed elsewhere (p. 228). In some cases of rickets the serum phosphate concentration may be within normal limits, the serum calcium being diminished (low calcium rickets). Under such circumstances the rachitic condition is commonly complicated by tetany (infantile tetany). It was pointed out by Howland and Kramer that if the concentration of calcium be multiplied by that of phosphate, each expressed in milligrams per 100 cc. of blood serum, a product is obtained which, in the normal child, ranges from 50 to 60. When this product is below 30, rickets is invariably present and when it is above 40 either healing is occurring or rickets has not been present. The serum alkaline phosphatase activity is increased, roughly in proportion to the severity of the condition (p. 250).

There can be little question but that vitamin D deficiency alone is responsible for the production of rickets in children. However, there is no precise information as to whether other dietary features that influence the development of this condition in certain experimental animals are also operative in man. For example, in the rat, the rachitogenic effect of D hypovitaminosis is influenced considerably by the quantities and proportions of calcium and phosphorus in the diet. At any given level of vitamin D deficiency, the development of rickets is facilitated by lowering the intake of these elements and by increasing the dietary Ca : P ratio above 2 : 1. A similar rachitogenic effect may be produced in the rat by administration of excessive amounts of cations which form insoluble, and therefore poorly absorbable phosphates, e.g., beryllium, lead, iron, aluminum, strontium, magnesium. Clinical experience suggests that factors which promote absorption of calcium or phosphorus from the intestine exert a beneficial influence in rickets, e.g., increased acidity of the intestinal contents, and absence of excessive amounts of phytic acid (p. 225).

Osteomalacia. The metabolic disturbance in osteomalacia is similar to that in rickets, both being usually dependent upon vitamin D deficiency. Diminution in the concentration of serum phosphate is one of the most constant features of this condition.

Idiopathic Steatorrhea. Under this designation are included conditions commonly termed celiac disease, sprue and nontropical sprue. The characteristic fatty diarrhea in these conditions is frequently accompanied by skeletal demineralization, dwarfism, low serum calcium and phosphorus, and characteristic manifestation of rickets and tetany. These abnormalities of calcium and phosphorus metabolism are generally regarded as secondary to the excessively large quantity of fat in the intestine and as due to defective absorption of calcium, phosphorus and probably vitamin D.

Hyperparathyroidism. The injection of parathyroid hormone is followed by a diminution in the concentration of serum phosphate which is perhaps a result of the increased elimination of phosphate in the urine (p. 245). Similar observations have been made in patients with hyperparathyroidism (osteitis fibrosa diffusa). The decrease in serum phosphate is roughly proportional to the degree of elevation of serum calcium in this condition. Following the repeated administration of large doses of parathyroid hormone, with a maintained serum calcium concentration between 15 and 20 mg. per 100 cc., the elimination of phosphate in the urine diminishes and the serum phosphate begins to rise. This effect upon serum phosphate is due not directly to the action of the parathyroid hormone but to the development of renal functional insufficiency which is also manifested at the same time by an increase in the concentration of the nonprotein nitrogenous constituents of the blood.

Fanconi Syndrome. This designation is applied to a group of conditions, exhibiting a familial tendency, characterized by inadequate renal tubular reabsorption of certain constituents of the glomerular filtrate due, presumably, to inherent defects in certain enzyme transport mechanisms in the tubular epithelial cells. This does not include functional derangements associated with morphologic tubular damage, e.g., nephrosis. The following substances, singly or in various combinations, are excreted in the urine in abnormally large amounts: glucose (p. 62); various amino acids (p. 183); lactic acid (p. 183); β-hydroxybutyric acid (p. 183); ammonia (p. 183); phosphate. There are frequently acidosis, hypophosphatemia, and rickets or osteomalacia. The serum calcium concentration is usually within normal limits and the serum alkaline phosphatase activity increased. We are concerned here only with the abnormality in phosphate. The other aspects are discussed elsewhere.

The increased urinary excretion of phosphate, due to its inadequate tubular reabsorption from the glomerular filtrate, is believed to be the direct cause of the lowered serum phosphate concentration. If this falls to a sufficiently low level, the Ca : PO_4 ratio becomes unfavorable for deposition of bone mineral, with the consequent development of rickets (infants and growing children) or osteomalacia (adults).

Hyperphosphaturia, hypercalciuria, hypophosphatemia, increased alkaline phosphatase, and rickets or osteomalacia may occur without any of the other characteristic features of the Fanconi syndrome in the typical form, e.g., aminoaciduria. The designation "tubular insufficiency without glomerular insufficiency" has been applied to this condition (a form of "renal tubular acidosis"). It has been suggested that the underlying defect may be an inadequate amount of phosphatase in the renal tubular cells. It has been reported that phosphatase activity in these cells is increased by vitamin D, and there are reports of improvement in several of the manifestations of the Fanconi syndrome following administration of this agent.

Increased Carbohydrate Utilization. As was mentioned above, the serum phosphate concentration diminishes during periods of increased carbohydrate utilization. The administration of insulin or epinephrine produces this effect apparently by favoring certain phosphorylation reactions (p. 5). Insulin produces this effect in both normal individuals and those with diabetes, but the action of epinephrine in this connection is dependent upon the presence of an adequate supply of insulin, the hypophosphatemic response being diminished in diabetes. Consistently subnormal serum phosphate concentrations may occasionally be observed in individuals suffering with hyperinsulinism.

ABNORMAL URINARY PHOSPHATE

One of the means by which the kidney aids in maintaining the acid-base balance resides in its ability to transform the basic phosphate (B_2HPO_4) of the blood to the acid phosphate (BH_2PO_4) which is eliminated in the urine. In the presence of acidosis or factors which tend to produce acidosis, the elimination of acid phosphate in the urine is increased, constituting one of the compensatory measures which aid in the maintenance of the acid-base balance (p. 329). This condition of phosphaturia is not observed in the acidosis of advanced nephritis, which is commonly associated with retention of phosphate as well as other constituents of the blood which are normally eliminated in the urine.

One of the first demonstrable effects of the administration of parathyroid hormone is an increase in the quantity of phosphate eliminated in the urine (p. 227). This is believed by some observers to be the cause of the subsequently occurring hypophosphatemia. Likewise, an increase in urinary phosphate has been observed in clinical hyperparathyroidism (osteitis fibrosa diffusa). In hypoparathyroidism (parathyroid tetany), on the other hand, the urinary phosphate is diminished in quantity. The urinary phosphate is diminished in rickets, the greater part of the ingested phosphate being excreted in the feces, owing perhaps in part to inadequate absorption. The administration of vitamin D or irradiation with ultraviolet light increases the urinary elimination of phosphate in rickets and osteomalacia.

The occurrence of hyperphosphaturia as a cardinal feature of the Fanconi syndrome is referred to in the discussion of abnormalities in serum phosphate (p. 244).

DISTURBANCES OF BONE FORMATION AND MINERALIZATION

Abnormalities may occur in both phases of bone construction, i.e., (*a*) in the formation of the osteoid matrix, and (*b*) in the subsequent process of mineralization. Accurate diagnosis and proper therapy require that these two classes of skeletal disorders be clearly differentiated.

Table 9. Osteoporosis

1. *Congenital*
    - Osteogenesis imperfecta
2. *Inadequate stimulation (stress and strain)*
    - Disuse atrophy
3. *Degenerative*
    - Senile osteoporosis
4. *Nutritional*
    - Malnutrition (especially protein)
    - Vitamin C deficiency
    - Vitamin A deficiency
5. *Endocrine*
    - Estrogen deficiency (postmenopausal)
    - Androgen deficiency (eunuchoidism)
    - Adrenal hyperfunction (Cushing's syndrome)
    - Hyperthyroidism
    - Hypothyroidism
    - Acromegaly
    - Panhypopituitarism (Simmonds' disease)
6. *Idiopathic*

Table 10. Osteomalacia

1. *Nutritional*
    - Vitamin D deficiency (rickets)
    - Calcium and/or phosphorus deficiency
    - Steatorrhea
    - Chronic diarrhea (ulcerative colitis)
    - Gastrocolic fistula
2. *Renal Functional Impairment*
    - Glomerular damage ("renal rickets")
    - Tubular malfunction
        - Fanconi syndrome
        - Renal tubular acidosis
3. *Endocrine*
    - Hyperparathyroidism

Defective osteoid formation, designated *osteoporosis* (Table 9), is not due to disturbance in calcium or phosphorus metabolism, and there is no consistent alteration in serum Ca or PO_4 in conditions in this category. The fundamental defects usually consist in inadequate protein synthesis (generalized), excessive protein catabolism, (generalized), or nutritional inadequacies more specifically affecting osteoid formation, (e.g., disuse atrophy, vitamin A deficiency, and defective osteoblastic function, e.g., scurvy, osteogenesis imperfecta). Inasmuch as these disorders are characterized by inadequate regenerative capacity of the bones, i.e., inadequate osteoblastic proliferation and/or function, there is no increase in serum alkaline phosphatase, which is a reflection of osteoblastic activity (p. 249).

On the other hand, in the conditions included under the designation, *osteomalacia,* the fundamental defect is in the metabolism of calcium and/or phosphorus (Table 10). There is no necessary abnormality in osteoid formation. Indeed, this is usually excessive, due to the increased stimulation resulting from weakening and injury of the bones. The nature of the metabolic aberration varies, but the effect in each instance is inadequate mineralization of the osteoid matrix. In these conditions, in addition to alterations in serum calcium and phosphorus, which are often quite characteristic, there is often an increase in serum alkaline phosphatase activity, a reflection of increased osteoblastic activity. This may be absent in conditions such as glomerulonephritis, due to the coexistence of serious impairment of protein nutrition. In general, however, increase in serum alkaline phosphatase is of great value in distinguishing osteomalacia from osteoporosis.

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CHAPTER VIII

Phosphatase Activity

Phosphatases are enzymes capable of hydrolyzing phosphoric esters, both aliphatic and aromatic, including a portion of the phosphoric esters of the circulating red blood cells and those present, in small amounts, in blood plasma. Originally demonstrated in rice and wheat bran, these agents, which hydrolyze phosphoric esters with the liberation of inorganic phosphate, have been identified in practically all tissues of a variety of animals. They have been demonstrated in the following situations: mucosa of the small intestine, colon and stomach, kidneys (cortex and medulla), ossifying cartilage, bone, periosteum, teeth, spleen, liver, pancreas, lungs, thyroid, thymus, suprarenal glands, testis, cerebrum, cardiac and skeletal muscle, milk, bile, urine, blood plasma, red blood cells and leukocytes. In the fetus and growing animal the greatest relative quantity of phosphatase is found in the bones and teeth. In the adult animal, the intestinal mucosa contains the greatest amount per unit of weight (wet tissue), being followed in approximate order in this regard by the renal cortex, whole bone, thyroid, spleen, lungs, suprarenal glands and pancreas, with some variation of this order in different species. It has also been found in the bladder, trachea, aorta and retina of different species of animals.

The phosphatase activity of the majority of tissues studied appears to be dependent upon the action of the same enzyme or, at least, enzymes possessing similar properties. The identity of the phosphatases of bone, intestinal mucosa, kidney and blood plasma has been fairly definitely established on the basis of the similarity of certain factors. Among these are the dissociation constant of the enzyme-substrate compound, the ratio of rates of hydrolysis of various esters, activation by magnesium and the optimum pH for hydrolytic activity. However, the phosphatase enzyme present in red blood cells is different from that in other tissues. Synthesis as well as hydrolysis of the phosphoric esters can be accomplished by phosphatase from bone, kidney, intestine, blood plasma and red blood cells under suitable conditions. The serum phosphatase, as well as that of most other tissues, is activated by a variety of substances, among which are magnesium, iron, manganese, cobalt, nickel, ascorbic acid and glycine. There is some evidence that this enzyme is inhibited by cholic acid, copper and zinc.

Four types of phosphomonoesterases of biological significance may be differentiated on the bases of their activity in different pH ranges. (1) a type (alkaline phosphatase) with optimum activity at about pH 9.3, present in blood plasma or serum, bone, kidney, intestine, mammary gland, spleen, lung, leukocytes, adrenal cortex and seminiferous tubules: (2) a type (acid phosphatase) with optimal activity at pH 6, occurring in mammalian erythrocytes and yeast; (3) a type (acid phosphatase) with optimum activity at pH 5, occurring in prostatic epithelium, spleen, kidney, blood plasma, liver, pancreas and rice bran; (4) a type (acid phosphatase), with optimal activity at about pH 3 to 4, obtained from taka-diastase.

From a clinical standpoint, interest is centered particularly upon the phosphatase activity of the blood plasma or serum, i.e., alkaline phosphatase, and acid phosphatase.

NORMAL SERUM ALKALINE PHOSPHATASE

According to the method of Bodansky, a unit of phosphatase activity is defined as "equivalent to the actual or calculated liberation of 1 mg. of phosphorus as the phosphate ion during the first hour of incubation at 37° C. and pH 8.6, with the substrate containing sodium beta-glycerophosphate, hydrolysis not exceeding 10 per cent of the substrate." Employing this procedure, the range ot normal values for plasma or serum alkaline phosphatase activity in adults is 1.5 to 4.0 units per 100 cc., that for children being 5 to 14 units. In our experience, values in adults of less than 7 units are of little clinical significance in hospital patients, in the absence of other significant abnormalities. The alkaline phosphatase activity of plasma is low at birth, rises rapidly to a maximum during the first month of life (14 units) and remains fairly high during the second year, the values in later childhood falling to within the normal adult range. Normal values obtained by the King-Armstrong method range from 3 to 14 units (adults).

There is some evidence that senility, malnutrition and anemia tend to lower the serum phosphatase activity; this should be taken into consideration in interpreting variations in this factor in clinical conditions. Some observers have found that high protein diets cause a decrease and high carbohydrate diets an increase in alkaline phosphatase activity of the serum; an increase occurs during alimentary hyperglycemia.

The alkaline phosphatase of normal plasma (or serum) originates mainly, if not exclusively, in the bones, where it is formed in large amounts by actively functioning osteoblasts (p. 229). Its possible role in bone mineralization is discussed elsewhere (p. 230). Despite the present state of uncertainty regarding the specific function of this enzyme in bone formation, measurement of serum alkaline phosphatase activity is of value clinically in the study of patients with diseases of two systems, viz., (1) the skeleton, and (2) the liver and biliary tract.

Skeletal Diseases

The clinical significance of serum alkaline phosphatase activity in skeletal disorders may be indicated by the following generalizations:

1. The enzyme originates in functioning osteoblasts.
2. An increase (serum) reflects osteoblastic proliferation or increased activity.
3. The usual stimulus to osteoblastic proliferation is bone stress, strain, weakness, or injury.

4. An increase in alkaline phosphatase activity usually implies:
    (*a*) Inadequate mineralization, or
    (*b*) injury, with
    (*c*) attempts at active repair, which implies
    (*d*) adequate bone matrix.
5. Therefore, an increase occurs in osteomalacia, not in osteoporosis (p. 246).

Rickets. One difficulty in the path of complete acceptance of the phosphatase hypothesis of calcification of bone is the fact that there is no deficiency in this factor in the zone of hypertrophic cartilage cells in rickets. It has been suggested that the fault may reside in the existing deficiency in plasma inorganic phosphate or in one possible substrate for bone phosphatase, the plasma phosphoric ester content. It is interesting in this connection to note that several observers have reported marked diminution in the phosphoric ester content of the red blood cells in human and experimental rickets and an increase following the administration of large quantities of vitamin D. This observation is of particular significance in the light of the finding that the ester content of the red corpuscles changes continuously with the growth of the child, rising steadily from birth, reaching a maximum during the second year and then falling gradually until the adult level is reached. It has also been found that the phosphoric ester content of whole blood is high during the period of most active bone development. These observations suggest that the ester phosphorus of the red blood cells may be the main circulating substrate or store of substrate for the bone enzyme.

The serum phosphatase activity is consistently considerably increased in active rickets. Serum phosphatase activity, normally 5 to 14 units per 100 cc. in children (Bodansky method), may be 20 to 30 units in mild rickets, as high as 60 units in marked rickets and over 60 in very marked rickets. Values as high as 190 units have been obtained in the last group. These figures may be regarded as reliable criteria of the severity of the condition at the time of first observation. Following the institution of antirachitic therapy the serum phosphatase activity decreases, usually after an interval of four to twelve days. A high normal figure may be reached within two months and is frequently maintained for some time during the period of active repair. When bone reconstruction is complete the serum phosphatase is within normal limits. It has been found that the plasma phosphatase activity may remain above normal even after healing is apparently complete roentgenographically. There is usually a reciprocal relationship between plasma phosphatase activity and inorganic phosphate concentration in rickets. In the opinion of most observers, determination of plasma phosphatase activity may be regarded as a reliable means of detecting latent and active rickets and may be accepted as an index of improvement in this condition.

Hyperparathyroidism (Generalized Osteitis Fibrosa Cystica). A moderate increase in serum phosphatase activity occurs in clinical and experimental hyperparathyroidism (p. 233). Values as high as 65 units have been reported, but the values usually range between 20 and 40 units. A gradual fall to normal levels has been observed following parathyroidectomy in such cases, associated with increasing calcification of the affected bones.

Osteitis Deformans (Paget's Disease). Increased serum phosphatase activity has been observed rather consistently in patients with osteitis deformans involving several bones. Higher values have been obtained in this condition than in any other clinical disorder. Bodansky and Jaffe found values of 15 to 125 units in ten cases of the polyostotic variety and 4.9 to 23.1 units in thirteen cases with localized involvement of one or two bones. Spontaneous healing by sclerosis was associated with relatively low values for serum phosphatase activity. Age appears to be a factor, the oldest patients showing the lowest values. The finding of moderately elevated serum phosphatase activity, together with normal values for serum calcium and inorganic phosphorus, is of distinct diagnostic significance in this condition. The degree of increase is also of prognostic value, reflecting the activity of the bone lesion, which can otherwise be gauged only by serial x-ray studies.

Miscellaneous Bone Disorders. Slightly elevated values for serum phosphatase activity (5–15 units) have been reported occasionally in patients with various forms of osteomalacia (p. 246), metastatic carcinoma involving bone, osteogenic sarcoma, healing fractures, Gaucher's disease with bone resorption, and osteosclerosis fragilis generalisata (marble bones).

Essentially normal findings are obtained in most cases of chronic arthritis, various forms of osteoporosis (p. 246), osteomyelitis, bone cysts, tumors not involving bone, achondroplasia, cretinism, calcinosis universalis, and multiple myeloma. Very high values are encountered occasionally in cases of carcinoma with extensive and widespread metastases involving the skeleton. Slight increases have been reported also in occasional cases of renal rickets and multiple myeloma. Decreased serum alkaline phosphatase activity has been reported in hypothyroid children and in scurvy.

ALKALINE PHOSPHATASE ACTIVITY IN JAUNDICE, HEPATIC AND BILIARY TRACT DISEASE

Obstructive and Hepatocellular Jaundice (p. 545). Increased serum phosphatase activity occurs in a large proportion of patients with both obstructive and hepatocellular jaundice (p. 546). Values as high as 60 units (Bodansky method) have been observed in such cases. Some believe that phosphatase activity tends to parallel the degree of hyperbilirubinemia in obstructive jaundice more closely than in hepatocellular jaundice, although this finding is by no means constant. Some authors feel that the determination of serum phosphatase activity may be of value in differentiating between these two types of jaundice. They believe that whereas in obstructive jaundice serum phosphatase activity and serum bilirubin concentration increase proportionately until the limit of phosphatase activity is reached (40–60 units), in hepatocellular jaundice the serum phosphatase rarely rises above 12 units in spite of a progressive increase in serum bilirubin.

The experience of the majority of observers is not in complete accord with this conclusion. It would appear that although phosphatase activity values above 20 units are obtained about twice as frequently in patients with obstructive jaundice as in those with hepatocellular jaundice, this factor is of distinctly limited value in differential diagnosis because of a wide overlapping of values in the two groups of cases. The following results were obtained in our laboratories: obstructive jaundice—less than 10 units (Bodansky), 32 per cent; 10–20 units, 26 per cent; 20–30 units, 35 per cent; more than 30 units, 7 per cent; hepatocellular jaundice —less than 10 units, 55 per cent; 10–20 units 32 per cent; 20–30 units 13 per cent. Moreover, normal serum phosphatase activity has been reported in infants with congenital atresia of the bile ducts. This subject is discussed elsewhere in more detail (p. 545ff.).

Other Biliary Tract Conditions. Many patients with portal cirrhosis present very high values for serum phosphatase activity with little or no increase in serum bilirubin concentration; others present normal phosphatase values with relatively high concentrations of serum bilirubin. Increased values for serum phosphatase activity may also be obtained in patients with metastatic carcinoma of the liver, the increase occurring in many instances before any demonstrable elevation of the serum bilirubin concentration. In the presence of known intra-abdominal malignancy, e.g., of the stomach, if skeletal metastases can be excluded, high serum alkaline phosphatase values suggest metastasis to the liver. No increase in serum phosphatase activity has been found in cases of hemolytic jaundice.

MISCELLANEOUS CONDITIONS

Increased serum phosphatase activity has been reported in the following conditions: during periods of calcification of hemorrhages in scurvy, with subsequent return to normal as the calcified areas are absorbed; active tuberculosis; chronic myeloid leukemia; Hodgkin's disease involving the bones; obstruction of the pancreatic duct; sarcoidosis; lymphatic leukemia (bone or liver involvement?).

SERUM ACID PHOSPHATASE

Normal plasma contains small amounts of acid phosphatase (pH 4.9). With the Bodansky procedure, the normal range is 0.5 to 1.5 units/100 cc. With the King-Armstrong procedure, normal values range from 1 to 3 units. Because of the presence of relatively large amounts of an acid phosphatase in red blood cells, care must be taken to avoid hemolysis in samples in which this determination is to be made. The acid phosphatase of normal plasma probably originates mainly in the liver and spleen. It is certainly not largely of prostatic origin, since it is present in the plasma of women and children in the same amounts as in normal men.

The chief, if not the only, clinical significance of serum acid phosphatase lies in its relation to the diagnosis of metastasizing prostatic carcinoma. Acid phosphatase activity is present in several organs, but in the adult prostate it is at least 100 times that in other tissues. It has been shown that this enzyme is formed by mature prostatic epithelial cells and is passed into the prostatic secretion and ultimately into the seminal fluid. Immature prostatic cells (i.e., preadolescent boy or castrate) do not form this enzyme, but do so under stimulation by androgens.

In a large proportion of cases of prostatic cancer, the malignant cells retain the capacity to form large amounts of this enzyme, even after they have metastasized to other tissues, e.g., periprostatic structures, bones. In these extraprostatic locations, the enzyme gains access to the blood stream and the serum acid phosphatase activity is increased. Inasmuch as no other tissue is capable of forming sufficient amounts of this enzyme to raise its concentration in the blood plasma to a high level, such increases may be regarded as presumptive, and at times conclusive evidence of the presence of prostatic cells outside the prostatic capsule, i.e., carcinomatous metastases. Although, theoretically, similar elevations may occur as a result of ulcerative lesions or surgical procedures involving the prostate, they are practically never observed under such circumstances. Experience has indicated: (*a*) that serum acid phosphatase values above 5 Bodansky units or 10 King-Armstrong units are not obtained in conditions other than metastasizing prostatic cancer; (*b*) that values of 3 to 5 Bodansky or 5 to 10 King-Armstrong units may be regarded as indicative of this condition in the absence of substantial evidence to the contrary; (*c*) that values of 1.5 to 3 Bodansky or 3 to 5 King-Armstrong units may be of diagnostic value under certain circumstances, e.g., they suggest metastasis in cases of known prostatic cancer.

In some cases of highly anaplastic carcinoma (i.e., very immature cells), especially in older men (i.e., little androgen production), the serum acid phosphatase may be normal in the presence of metastases. Under such circumstances, the injection of 25 mg. of testosterone propionate daily for five days may stimulate production of the enzyme by these cells (Sullivan test). Significant increase in serum acid phosphatase after such treatment may be of diagnostic value.

Abnormally high values (as high as 700 units per 100 cc.) have been obtained in about 85 per cent of cases of metastasizing carcinoma of the prostate and normal values in about 95 per cent of cases of nonprostatic disease, including skeletal and other disorders accompanied by high values for serum alkaline phosphatase. A slight increase may occur in advanced Paget's disease (osteitis deformans), osteopetrosis, hyperparathyroidism, and carcinoma of the breast with extensive skeletal metastases (non-prostatic acid phosphatase.)

In addition to its usefulness in diagnosis, this determination may be of value in connection with treatment of prostatic cancer. In the majority of cases, castration is followed promptly (24–48 hours) by a sharp drop (av. 55%) in a previously elevated serum acid phosphatase level, the decline continuing for several months, falling in favorable cases to essentially normal values. Similar, but less dramatic results are obtained with estrogen therapy. This type of response reflects a satisfactory therapeutic effect, and is of prognostic value. Recurrence or progression of the lesion, after a period of remission, is often reflected in a rise in serum acid phosphatase before other clinical manifestations appear.

The significance of an increase in this factor may be summarized as follows:

1. It corroborates the diagnosis of metastatic prostatic cancer, suggested by other methods of examination.
2. It provides evidence of metastasis before this is demonstrable otherwise.
3. It indicates the prostatic origin of metastatic lesions when the primary tumor may not be detectable clinically.
4. It detects recurrence or progression after a period of quiescence.
5. It aids in the selection of cases suitable for castration or estrogen therapy.

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CHAPTER IX

Magnesium Metabolism

ABSORPTION AND EXCRETION

The absorption of magnesium from the bowel resembles that of calcium in many respects. An excessively high intake of fat, phosphate, calcium and alkalis appears to diminish the absorption of magnesium from the upper intestine. It is of interest also to note that a high magnesium intake appears to increase calcium elimination in the urine. The influence of the above mentioned factors in this connection is probably dependent upon their influence on the solubility of magnesium salts. No extensive data are available regarding the effects of vitamin D upon magnesium absorption. The fact that the magnesium content of the bones is not decreased and is usually actually increased in rickets argues against the importance of vitamin D in this connection.

Like calcium, magnesium is excreted in the feces and urine. Under normal conditions, about 50 to 80 per cent is excreted by the bowel (bile and intestinal secretion) and the remainder by the kidneys. After parenteral administration of magnesium salts, 70 to 90 per cent is excreted in the urine in the presence of normal renal function. The administration of acidifying substances, such as ammonium chloride and calcium chloride, is followed by an increased urinary elimination of magnesium, as of calcium. On the other hand, hyperthyroidism and thyroid administration, which cause an increase in urinary calcium, are practically without effect upon magnesium excretion. The urine magnesium increases slightly and temporarily after administration of parathyroid hormone, but then returns to normal. It is also within normal limits in clinical hyperparathyroidism. After correction of this condition by parathyroidectomy, there may be a temporary fall in magnesium excretion in the urine. There is evidence that the urinary excretion of magnesium is interfered with in the presence of renal functional impairment.

BLOOD MAGNESIUM

Magnesium is present in both red corpuscles and plasma. In contrast to calcium, the concentration in the red cells is apparently considerably higher than in the plasma. Perhaps because of variations in the methods employed and the limited number of cases studied, there is still disagreement regarding the normal range of variation in blood magnesium concentration. The normal range of serum magnesium concentration is 1.8 to 3.6 mg. per 100 cc. in normal adults. Ultrafiltration and diffusion experiments indicate that 70 to 85 per cent (average 80%) of the serum magnesium is in a diffusible state, the nondiffusible fraction being probably combined with the serum protein as in the case of the nondiffusible fraction of serum calcium. The magnesium content of cerebrospinal fluid is higher than that of blood serum, averaging about 3.3 mg. per 100 cc.

Little is known regarding the factors involved in the regulation of the magnesium content of the blood. It is relatively unaffected by phosphate, protein, vitamin D or parathyroid hormone, although the last apparently causes a slight and transient elevation of serum magnesium. There is in some respects a reciprocal relationship between magnesium and calcium in the serum; e.g., in oxalate poisoning the decrease in serum calcium is accompanied by an increase in magnesium, while the hypermagnesemia induced by parenteral administration of magnesium salts is accompanied by a fall in serum calcium, at times to tetanic levels.

Abnormal Serum Magnesium. Because of the occurrence of marked variation in red blood cells, studies of magnesium in the blood in pathologic states have been confined largely to the determination of its concentration in the serum.

The general opinion is that significant deviations from the normal do not occur consistently enough to be of distinct clinical importance. Slightly increased values have been reported in various chronic infections, atherosclerosis, hypertrophic arthritis, oxalic acid poisoning, essential hypertension, and glomerulonephritis. Values as high as 13 mg. per 100 cc. have been reported following the ingestion of large quantities of magnesium sulfate by patients with chronic glomerulonephritis and renal functional impairment. It is believed by some that the degree of magnesium retention under such circumstances may contribute to the development of coma which may be mistakenly regarded as uremic in nature. Many observers have failed to confirm these observations. However, it would appear that elevated values for serum magnesium may occur more consistently in chronic glomerulonephritis than in any other clinical disorder in which this factor has been studied. Values of 3 to 6 mg. per 100 cc. have been reported frequently. A slight and very transitory increase in serum magnesium concentration may occur following the injection of parathyroid hormone. This increase usually precedes demonstrable changes in calcium and phosphorus metabolism produced by this agent. Some observers have reported a decrease under the same conditions.

Low values for serum magnesium have been reported in uremia, normal and abnormal pregnancy, rickets, hypervitaminosis B, marked experimental magnesium deprivation, a form of tetany unassociated with disturbances in calcium and phosphorus metabolism, and in epilepsy. However, many of the figures regarded by individual authors as subnormal fall within the range regarded as normal by others. In fact, with few exceptions, the same statement may be made regarding the great majority of all reported abnormal values for serum magnesium concentration.

An increase in the nondiffusible fraction of serum magnesium (25–60% of the total) has been reported in hyperthyroidism, normal values (15–30% of the total) being restored by adequate treatment with iodine or subtotal thyroidectomy. Very low values for nondiffusible magnesium, below 5 per cent of the total, and at times 0 per cent, have been obtained in patients with myxedema, an increase occurring after administration of thyroid extract. The significance of these changes is not known.

CHAPTER X

Iron Metabolism

Although the amount of iron in the body is small (about 45 mg./kg. body weight), the fact that it is an essential constituent of hemoglobin and of cytochrome and other components of respiratory enzyme systems (cytochrome oxidase, catalase, peroxidase), makes it an element of great fundamental importance. Its chief functions lie in the transport of oxygen to the tissues (hemoglobin) and in cellular oxidation mechanisms (cytochrome system).

ABSORPTION AND EXCRETION

Iron differs from practically all other electrolytes in that the quantity in the body is controlled by regulation not of its excretion, but rather of its absorption into the organism. The body stores of iron are conserved very efficiently, only minute amounts being excreted in the urine and feces, usually less than 1 mg. daily. Relatively large amounts are, of course, lost in the menstrual flow. That present in the body in various substances, e.g., hemoglobin, is almost completely reutilized following its liberation in the course of metabolic degradation of these substances. The bulk of the iron of the feces is unabsorbed food iron; a very small amount enters the intestine in the bile and escapes in the feces.

Several factors make absorption of iron difficult, regardless of the form in which it is ingested: (*a*) the relatively high pH in the jejunum favors the formation of insoluble basic iron compounds; (*b*) iron salts of bile acids are relatively insoluble; (*c*) the presence of relatively large amounts of phosphate favors the formation of insoluble iron phosphates; (*d*) absorption of iron is interfered with, therefore, in the absence of free HCl in the stomach (achlorhydria) and by administration of alkalis.

Absorption occurs chiefly in the upper duodenum and stomach, but, to a lesser extent, throughout the small intestine. Absorption of inorganic salts of iron compares favorably with that of the iron of foodstuffs, which is chiefly a colloidal ferric hydroxide. In general, ferrous iron is better absorbed than ferric.

Normal gastric acidity facilitates the ionization and solution of ingested iron and delays the formation of insoluble, undissociable compounds which may form above pH 5. At the slightly acid reaction usually encountered in the duodenum, ionizable iron compounds are converted chiefly to ferrous or ferric hydroxides, the former being favored at higher acidities and by the presence of reducing agents in the digestive mixture (glutathione, ascorbic acid, SH-groups of proteins and digestion products).

The normal organism absorbs iron only in proportion to its needs, the quantity absorbed being determined by the magnitude of the body reserves of this element. Apparently the gastrointestinal mucosa is the tissue immediately responsible for its acceptance or rejection, which is regulated by the amount of ferritin in the mucosal epithelial cells. This is an iron-protein complex (23% Fe), containing aggregates of ferric hydroxide and a protein, apoferritin (m.w. 450,000). Apoferritin has vasodepressor and antidiuretic properties. Ferritin occurs also in other parenchymal cells, e.g., the liver and spleen, and in the bone-marrow and reticuloendothelial cells generally. It is believed to be the chief storage form of iron.

Ferrous iron, on entering the mucosal epithelial cell, is oxidized, forming ferric hydroxide, which combines with the protein, apoferritin, synthesized in the cell, forming ferritin (Fig. 25). Apoferritin is present in very small amounts and is apparently unstable, breaking down quickly unless iron is present to convert it to ferritin, which is more stable. The equilibrium between these intracellular forms of iron is potentially reversible. Entrance of iron into the cell ceases when its capacity for storing ferritin is exhausted. The amount of ferritin in the mucosal cells therefore regulates iron absorption from the gastrointestinal tract.

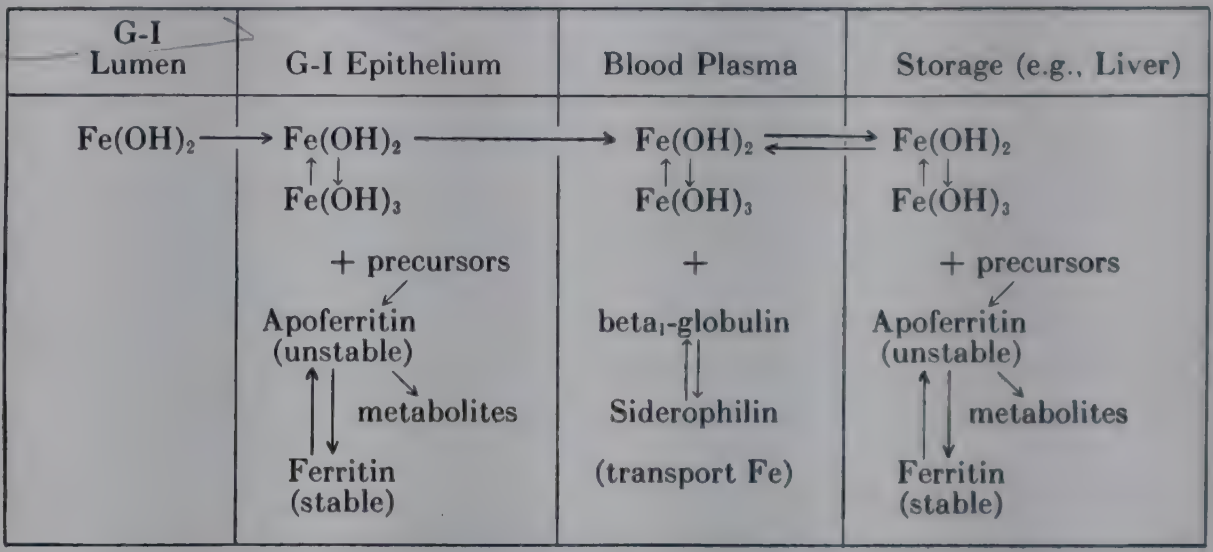
G-I Lumen
G-I Epithelium
Blood Plasma
Storage (e.g., Liver)
Fe(OH)₂
Fe(OH)₂
Fe(OH)₃
+ precursors
Apoferritin (unstable)
metabolites
Ferritin (stable)
Fe(OH)₂
Fe(OH)₃
+
beta₁-globulin
Siderophilin
(transport Fe)
Fe(OH)₂
Fe(OH)₃
+ precursors
Apoferritin (unstable)
metabolites
Ferritin (stable)

Fig. 25. Mechanism of absorption, transport, storage, and mobilization of iron.

TRANSPORT

In accordance with body needs, the reactions in the mucosal cell, outlined above, are reversed, the mechanism being unknown. It has been suggested that slight decrease in circulating Hb, with a fall in O_2 tension, may favor reduction of ferric to ferrous iron, causing breakdown of ferritin to apoferritin and permitting absorption of additional iron. The ferrous iron thus mobilized passes into the blood plasma, undergoes reoxidation to the ferric state and combines with one of the plasma beta-globulins. This compound, termed "siderophilin," is present in a concentration of about 0.25 gm./100 ml. plasma. The iron content of plasma is 80 to 170 µg./100 ml.; this is the transport iron (ferric).

The iron content of whole blood is normally 40 to 60 mg./100 ml., averaging

45 in women and 52 in men. Practically all of this is in organic form, as hemoglobin, which contains about 0.335 per cent iron (ferrous), all of which is in the red blood cells.

After ingestion of inorganic iron, the plasma iron concentration increases three- to fourfold in about two to four hours, falling subsequently over a period of six to twelve hours, depending upon the amount ingested. The plasma appears to be the medium of transportation of iron from the intestine to the tissues in which it is utilized and stored. Absorbed iron is transferred to erythrocytes with remarkable rapidity, radioactive iron having been demonstrated in red blood cells in anemic dogs within a few hours after its ingestion. The plasma iron is influenced by and may be regarded as an index of (*a*) the quantity of iron absorbed from the intestine, (*b*) the adequacy of the tissue iron reserves, (*c*) the capacity of the bone marrow to utilize iron for hemoglobin synthesis, and (*d*) the activity of hemolytic processes.

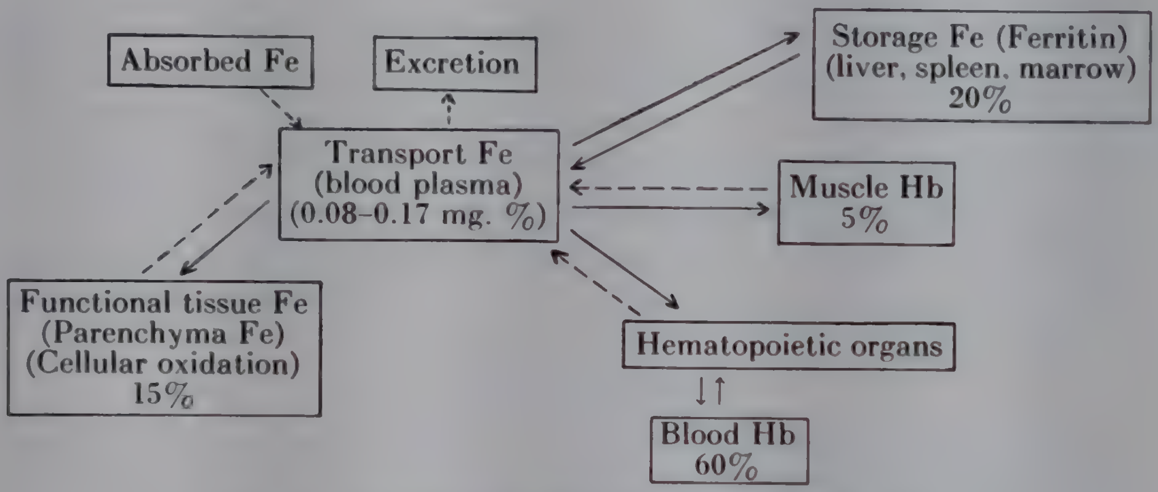

Fig. 26. Approximate distribution of iron in the body.

UTILIZATION; STORAGE

In the tissues, as needed, the plasma iron is apparently released from siderophilin, passes out of the capillary and into the cells, where it may be utilized or stored (as ferritin). The storage mechanism is identical with that described for the formation of ferritin in the gastrointestinal mucosal cells. The liver, spleen and intestinal mucosa are the chief storage sites, but other organs (e.g., pancreas, adrenals), and all reticuloendothelial cells contain ferritin. When iron is deposited in abnormally large amounts, hemosiderin may be formed, a compound similar to ferritin, but containing more iron (up to 35%).

Iron is utilized chiefly in the synthesis of hemoglobin, myoglobin (muscle Hb), and certain respiratory enzymes (cytochromes, peroxidase, catalase). The latter are probably formed in all cells, myoglobin in muscle cells, and hemoglobin in the developing red-blood cells (normoblasts, reticulocytes) in the erythropoietic tissues, principally the bone-marrow in man. The approximate distribution of iron in the body is as follows (Fig. 26): (1) Circulating Hb, 60 per cent, (2) myoglobin, 5 per cent; (3) storage iron, 20 per cent; (4) functional tissue iron (respiratory enzymes), 15 per cent. Thus, about 65 per cent of the body iron is in the form of hemoglobin, 20 per cent is in storage form, available for utilization, and 15 per cent is functional tissue iron, not readily available for other purposes. The iron of erythrocyte hemoglobin is readily available, but not that of myoglobin.

The quantity of iron mobilized in the organism daily far exceeds the exogenous supply. About 0.8 per cent of the circulating erythrocytes undergo disintegration daily (life 125 days), liberating about 7 to 8 gm. of Hb (0.34% Fe). In the course of its degradation, this gives rise to about 25 mg. of iron, the bulk of which is immediately utilized in the resynthesis of Hb, but some may enter the blood plasma for transport to other tissues. Synthesis of Hb, and consequently utilization of Fe for this purpose, require among other things adequate amounts of pteroylglutamic acid and vitamin B_{12}.

Mobilization of iron from ferritin in storage sites, e.g., liver, is accomplished by reversal of the reactions involved in its storage. As in the case of the gastrointestinal epithelium, this may involve initial reduction of ferric to ferrous iron, the latter passing out of the cell into the blood stream; this disturbs the intracellular iron equilibrium, ferritin breaking down to iron and apoferritin. The latter, being unstable, continues to break down until equilibrium is restored and loss of iron from the cell ceases. The reaction can then be reversed in the direction of ferritin formation.

REQUIREMENT

Since only minute amounts of iron are excreted, the exogenous requirement should be correspondingly low. However, it is believed that adults require 5 to 15 mg. daily to meet ordinary and unanticipated demands; perhaps 15 to 20 mg. is more satisfactory for women (menstruation, pregnancy, lactation). Young children (4 to 8 years) require about 0.6 mg./kg. body weight and infants (to 1 year) 1 to 2 mg./kg.

ABNORMAL IRON METABOLISM

Disturbances in iron metabolism are evidenced by (*a*) decreased formation of hemoglobin, (*b*) decrease in circulating hemoglobin, (*c*) abnormalities in the serum iron concentration or (*d*) abnormal deposition of iron-containing pigment (hemosiderin) in the tissues. A consideration of the pathogenesis of various types of anemia is beyond the scope of the present discussion, but it may be stated that certain forms of hypochromic and microcytic anemia are dependent primarily upon inadequate supply or absorption of iron, the latter occurring particularly in the presence of gastric anacidity. Absorption is decreased also in animals with severe subcutaneous infection (abscess). Iron deficiency may also result from hemorrhage, with exhaustion of the available tissue reserves of this element. Low values for plasma or serum iron have been reported in hemorrhagic and hypochromic types of anemia. High values occur in forms of anemia characterized by diminished hemoglobin formation not due to iron deficiency, as in pernicious anemia.

Deposition of excessive amounts of hemosiderin in the tissues occurs (*a*) as a result of excessive breakdown of erythrocytes in hemolytic types of anemia, (*b*) in conditions in which there is inadequate synthesis of hemoglobin due to factors other than iron deficiency (pernicious anemia) and (*c*) in hemochromatosis, the cause of which is unknown. In the last condition, enormous amounts of iron may be deposited in the tissues, especially the liver, pancreas and retroperitoneal lymph nodes, over 50 gm. having been found in the body (exclusive of the blood) in some cases. Studies of iron balance have revealed no significant abnormality in this condition, but it is possible that a slight degree of abnormal retention may exist over a period of many years. Studies of serum iron in hemochromatosis have yielded equivocal results, but recent observations suggest that significant abnormalities may occur rather consistently. In one series, the serum iron concentration ranged from 144 to 282 μg./100 cc. (normal 80–170 μg.). Moreover, whereas the iron-binding capacity of the plasma proteins of normal subjects averages about 200 μg./100 cc., that of patients with hemochromatosis averages only about 25 μg. Calculated on this basis, the capacity of the plasma to bind iron is apparently saturated to the extent of about 90 per cent in this disease, as contrasted to the normal 30 to 35 per cent saturation. Another reflection of this abnormal situation is the fact that administration of iron is followed by a much smaller rise in serum iron in patients with hemochromatosis than in normal subjects.

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CHAPTER XI

Sulfur Metabolism

Sulfur is a nutritionally essential element, occurring in substances of physiologic importance, including thiamine, insulin, anterior pituitary hormones, glutathione, cysteine, methionine, and taurocholic acid. However, indications for and methods of investigation of disturbances of sulfur metabolism are so limited that this subject requires only brief discussion here.

ABSORPTION

Sulfur is ingested in (1) inorganic (Na, K and Mg sulfates) and (2) organic forms. The latter may be subdivided into (*a*) nonprotein sulfur (sulfolipids and sulfatides) and (*b*) protein sulfur (the sulfur-containing amino acids, cystine and methionine; glycoproteins, such as mucoitin-sulfuric acid in mucin and ovomucoid and chondroitin-sulfuric acid in cartilage and tendons). The most important source of sulfur is the cystine and methionine of ingested proteins. Inorganic sulfate is absorbed as such from the intestine into the portal circulation, as are the amino acids, cystine and methionine, liberated by digestion of protein. A small amount of sulfide may be formed in the bowel by the action of bacteria but, if absorbed into the blood stream, this is rapidly oxidized to sulfate.

INTERMEDIARY METABOLISM

Sulfur reaching the liver in the portal blood undergoes the following changes:

(1) A portion of the organic S escapes oxidation. Some of this fraction is utilized for the formation of S-containing substances, such as insulin, anterior pituitary hormones, taurocholic acid, melanin, glutathione. The remainder is excreted in the urine as neutral S.

(2) The bulk of the organic S is oxidized in the liver to inorganic sulfate. A portion of the latter, together with a portion of the inorganic sulfate absorbed from the intestine, enters the systemic circulation and is excreted in the urine.

(3) A portion of the inorganic sulfate is combined in the liver with various phenol derivatives, such as indole, formed in the bowel largely by bacterial decomposition of protein, to form ethereal sulfates, which are excreted in the urine. In addition to the participation of inorganic sulfate in this detoxication process, the amino acid cysteine (formed from cystine) may form conjugation products (mercapturic acids) with certain toxic compounds, such as brombenzene.

SULFUR IN BLOOD

The normal concentration of sulfur in the blood serum is as follows: inorganic S, 0.5 to 1.1 mg. per 100 cc.; ethereal sulfate S, 0.1 to 1.0 mg. per 100 cc.; neutral S, 1.7 to 3.5 mg. per 100 cc. Whole blood contains 2.2 to 4.5 mg. of neutral S per 100 cc., the higher value being due in large measure apparently to the presence of thioneine and glutathione in the blood cells. The serum sulfate concentration may be increased in the presence of renal functional impairment, pyloric and intestinal obstruction, and leukemia. Marked sulfate retention in advanced glomerulonephritis may contribute to the development of acidosis. An increase in the blood indican concentration (indoxyl potassium sulfate) may occur in uremia (normal, 0.026–0.085 mg. per 100 cc.).

EXCRETION

Sulfur is excreted in the urine in the three forms in which it exists in the blood. The total S output, since it is derived chiefly from protein under ordinary circumstances, varies with the protein intake and the extent of tissue protein catabolism, averaging about 2.5 gm. (as SO_3) daily under normal conditions. The urinary $N : S$ ratio normally ranges from 13 to 16 in the fasting state or when meat constitutes the bulk of the protein intake.

The normal total sulfate content (inorganic plus ethereal sulfates) of the urine on an average mixed diet ranges from 1.5 to 3 gm. daily (as SO_3), comprising 85 to 95 per cent of the total S output. The proportion as well as the absolute amount excreted varies directly with the protein intake. The total sulfate excretion may be diminished in the presence of renal functional impairment and is increased in conditions accompanied by excessive tissue protein breakdown, such as high fever and increased metabolism.

Inorganic sulfate (Na, K, Ca, Mg and NH_4 sulfates) normally comprises 85 to 95 per cent of the total sulfate of the urine, the remainder (5–15 per cent) being ethereal sulfate, which normally ranges from 0.1 to 0.25 gm. daily (as SO_3). This fraction consists of sodium and potassium salts of phenolic sulfuric acid compounds (e.g., indoxyl potassium sulfate or indican) and therefore varies with the quantity of phenolic substances produced in the intestine or otherwise entering the body. Increase in urine ethereal sulfate (indican) (absolute and relative) may occur in intestinal obstruction (intestinal stasis and increased putrefaction), paralytic ileus, generalized peritonitis, typhoid fever, and in association with bacterial decomposition of tissue protein and purulent exudates, as in gangrene, empyema, pulmonary suppuration, tuberculosis with cavitation and secondary infection. The greatest clinical importance of urine sulfate partition studies lies in the demonstration by this means of abnormal absorption of benzene and the control of industrial exposure to this agent. Benzene is excreted in the urine in the form of an ethereal sulfate (conjugated in the liver), the absorption of abnormally large amounts being reflected in a decrease in the proportion of total sulfate excreted as inorganic sulfate (below 70–80% of the total urine sulfate). In acute cases these values may fluctuate rather rapidly, e.g., from 8 to 10 per cent immediately after exposure to 75 to 80 per cent two days later.

The neutral S of the urine, normally comprising about 5 per cent of the total sulfur, consists of such substances as cystine, urochrome, taurine and its derivatives, thiocyanate, and thiosulfate. This fraction is increased in cystinuria (p. 184), melanuria (p. 185), and some cases of obstructive and hepatocellular jaundice (excretion of taurocholic acid in the urine) (p. 536).

CHAPTER XII

Iodine Metabolism

The metabolism of iodine is of particular significance in connection with the formation of thyroid hormone. Normal adults require about 25 μg. daily but the recommended optimal daily intake for adults is 100 to 200 μg., for children 50 μg. and for infants 20 to 40 μg. The requirement is probably increased during pregnancy. The iodine of most common foodstuffs is apparently readily available to the organism but is not superior to inorganic iodides. Because of the high iodine content of sea water, marine vegetation, sea foods, and vegetables and fruits grown on the seaboard are particularly rich in this element. Plants (and animal tissues) grown far inland, especially at high altitudes, may be deficient in iodine because of its low concentration in the water. In such regions, iodide is commonly added to the drinking water or table salt in concentrations of 1 : 5000 to 1 : 200,000 to ensure an adequate intake and to prevent the development of simple goiter (iodine-deficiency goiter).

ABSORPTION AND EXCRETION

Iodine and iodides can be absorbed from any portion of the alimentary tract, most readily perhaps from the small intestine, free iodine and iodates probably being first converted to iodide. Organic iodine compounds, e.g., diiodotyrosine and thyroxine, are in part absorbed as such and in part broken down in the stomach and intestines with the formation of iodides. Absorption can occur from other mucous membranes, the lungs, and the skin.

Iodine is excreted by the kidneys, liver, skin, lungs and intestine, and also in milk and saliva. It is almost entirely in the form of inorganic iodide, and may be either endogenous (including liberation from degradation of thyroxine and diiodotyrosine) or exogenous (food, water). Thyroxine is not demonstrable in excreta except after administration.

The quantity excreted normally varies with the intake. About 40 to 80 per cent is usually excreted in the urine (20–70 μg. daily in adults; 20–35 μg. in children). The urinary fraction of the total elimination is largest when the intake is lowest. Urine iodine is increased by exercise and other factors that increase metabolism (increased rate of turnover of thyroxine), except in the event of profuse sweating, when relatively large amounts are lost by this route. Urine iodine increases during pregnancy.

Iodine in the feces (2–11 μg. daily; 3–27% of intake) is almost entirely exogenous (unabsorbed food iodine), the remainder being derived from the bile and intestinal secretions. Bile contains 4 to 14 μg./100 ml. in the fasting state and about 50 μg./100 ml. after eating. A portion of this is reabsorbed in the intestine (enterohepatic circulation). Biliary iodine is chiefly of alimentary origin (exogenous) but is also in part endogenous (degradation of thyroxine in liver).

Under ordinary atmospheric conditions, negligible amounts of I are excreted by the skin. With profuse perspiration (heat, humidity, exercise), as much as 30 to 60 per cent of the total may be lost by this route. The amount in expired air also varies enormously, but may be as much as 10 to 30 μg. daily. The quantity present in milk varies with the iodine intake and may be increased by administration of iodine. Saliva contains 0 to 350 μg./100 ml., also depending on the intake.

BLOOD IODINE

Practically all of the iodine in the blood is in the plasma, the concentration in the resting state being 4 to 10 μg./100 ml. plasma (or serum). If no iodine has been administered for several days, less than 30 per cent of the plasma iodine is in inorganic form (iodide), practically all (4–8 μg./100 ml.) being bound to protein and precipitated with the latter by protein-precipitating agents. It is believed to be largely or entirely in the form of thyroxine. Measurement of the amount of this protein-bound iodine (PBI), reflecting the concentration of circulating thyroid hormone, is therefore of value in the clinical evaluation of the state of thyroid function.

In the fasting state the plasma inorganic iodide is quite constant even in the face of wide variations in thyroid activity (which affect only the plasma PBI) but increases strikingly after iodine is ingested, applied to the skin, or administered for purposes of cholecystography or urography.

DISTRIBUTION AND INTERMEDIARY METABOLISM

The body normally contains about 20 to 30 mg. of iodine, which is distributed approximately as follows: muscles, 50 per cent; thyroid, 20 per cent; skin, 10 per cent; skeleton 6 per cent. The concentration in the thyroid (10–40 mg./100 gm.) is higher than in other tissues (e.g., 0.03 mg./100 gm. muscle). The amount in all tissues diminishes when the intake is lowered, but the normal thyroid retains its capacity for trapping and storing iodine even under such circumstances. One of the important initial phases of the formation of thyroid hormone involves this remarkable capacity of thyroid cells for trapping and concentrating iodine, which is then rapidly utilized for synthesis of thyroxine.

The normal adult thyroid contains 2 to 28 mg. of iodine (0.1–0.55% dry weight), varying with the iodine intake and state of thyroid function. By far the largest part is in organic combination, stored in the follicular colloid as "iodothyroglobulin," a composite protein molecule containing thyroxine and diiodotyrosine in peptide combination. On demand, thyroxine is mobilized from this compound and is passed into the systemic circulation. It undergoes metabolic degradation apparently chiefly in the liver, its iodine component being excreted in the bile largely as inorganic iodide, but also in some organic combination (thyroxine metabolites).

The liver usually contains 0.5 to 2.3 mg. of iodine, exhibiting wide variations during periods of absorption and excretion. Iodine entering the portal vein from the intestine in part passes through the liver into the systemic circulation and is in part removed by the hepatic cells and excreted in the bile. Organic compounds containing iodine, exogenous or endogenous, are partly or completely degraded in the liver, most of the iodine being liberated as iodide.

IODINE METABOLISM AND THYROID HORMONE

The thyroid gland contains an iodized protein, "thyroglobulin," characteristically present in the colloid of the thyroid follicles, which apparently is the "storage form" of the thyroid hormone. Hydrolysis yields two iodine-containing compounds, related to tyrosine, viz., diiodotyrosine (about 70%) and thyroxine (about 30%), both of which exhibit thyroid hormone activity, and possibly a third, monoiodotyrosine, which has not been studied adequately. Diiodotyrosine is believed to be the biological precursor of thyroxine, which has been synthesized in vitro.

A number of substances have been shown to exhibit thyroid hormone activity (relief of human myxedema), all of which contain iodine substituted in the inner aromatic ring of a compound termed "thyronine" (4–4′–hydroxyphenoxy)-phenylalanine. A low order of thyroid activity has been produced with bromine compounds.

Thyroxine contains 1.8 per cent N and 65.4 per cent I (4 iodine atoms); diiodotyrosine, with about 4 per cent of the biological activity of thyroxine, contains 3.2 per cent N and 58.7 per cent I (2 iodine atoms). The naturally occurring forms of these substances are levorotatory; synthetic thyroxine shows no optical rotation, being a mixture of D- and L-forms (racemic), the former being apparently biologically inactive. There is evidence that thyroxine may undergo partial deiodination in the tissues to the more active triiodothyronine.

BIOSYNTHESIS AND SECRETION OF THYROID HORMONE

The function of the thyroid in the formation and secretion of thyroid hormone may be divided into six phases: (1) Entry of inorganic iodide from the circulation. (2) Concentration of inorganic iodide in the gland. (3) Synthesis of thyroglobulin in follicular cells. (4) Storage of thyroglobulin in colloid. (5) Liberation of thyroxine from thyroglobulin. (6) Passage of thyroxine into the circulation.

Accumulation of Iodine in Thyroid. Inorganic iodide, present in the blood plasma in low concentration (< 2 µg. %, except after administration), is actively removed by the acinar cells of the thyroid, which are able to "trap" iodine in enormously higher concentration (several hundred times) than that in the circulation. Under physiological conditions, inorganic iodide comprises only about 1 per cent of the total iodine of the thyroid gland and, in 15 minutes after administration of tracer doses of I^{131} to rats, over 90 per cent of the radioactivity in the gland is organically bound. This suggests that the selective accumulation of this element by the thyroid is related to the ability of the acinar cells to incorporate iodine in organic compounds. However, after administration of large doses of iodide or certain antithyroid agents (p. 269), which inhibit synthesis of thyroid hormone, inorganic iodide may accumulate in the gland in high concentration, indicating the presence of a mechanism independent of that involved in the organic conversion reactions.

Accumulation of iodine in the thyroid is accelerated and increased by administration of thyrotrophic hormone (TSH) and is markedly reduced by thiocyanate (p. 268).

The thyroid contains inorganic iodide, monoiodotyrosine, diiodotyrosine, thyroxine, and at least two as yet unidentified iodine-containing compounds. Almost all (about 97%) of the iodine in the gland is firmly bound to protein (thyroglobulin), the organic compounds mentioned above being liberated by hydrolysis. Free thyroxine (possibly loosely linked to protein), although present in small amounts (1.5% of the total I), nevertheless exists in over 100 times the concentration of protein-bound iodine in the plasma.

Synthesis of Thyroid Hormone. Although all details have not been definitely established, present evidence supports the following sequence of events.

Oxidation of Iodide. Iodide ion in the follicular cell undergoes oxidation to I_2 (elemental iodine) or to IO^-. It has been suggested that a peroxidase enzyme may be involved in this process. There is also evidence that the cytochrome-cytochrome oxidase system is involved. It seems likely that the transition from iodide, through an oxidized form, to incorporation in the benzene ring of tyrosine, as indicated below, may occur almost as a single step.

Iodination of Tyrosine and Coupling. Tyrosine is iodinated, probably first to monoiodotyrosine, then to diiodotyrosine. This process may occur at the level of protein molecules since, in vitro, diiodotyrosine and thyroxine are formed more readily by iodination of protein than of the free amino acid, tyrosine. Two molecules of diiodotyrosine (tyrosyl radicals) then undergo oxidative coupling, with the elimination of one alanine side chain, to form thyroxine (thyroxyl radical).

These I-binding and coupling reactions are accelerated by TSH and are inhibited by certain antithyroid agents (e.g., thiocarbamides; aminobenzenes) (p. 269). Thyroxine and its two precursors are incorporated in the protein known as thyroglobulin, which is passed into the colloid material in the lumen of the thyroid follicles, where it is stored. As indicated above, the processes of iodination and coupling of tyrosyl radicals may occur within the intact protein molecule.

Under physiological conditions there is little diurnal variation in the iodine content of the thyroid, the uptake of I and release of I-containing hormone occurring simultaneously, continuously, and at approximately constant rates.

Secretion of Thyroid Hormone. Present evidence favors the view that, in accordance with demand, probably under stimulation by TSH, the thyroglobulin in the gland undergoes hydrolysis by a proteolytic enzyme or enzymes, with release of its iodinated compounds, chiefly thyroxine. The latter, uncombined in peptide linkage, has been detected in thyroid tissue in about 100 times its concentration in the plasma. It is probable that this is the form in which the hormone is secreted, passing from the colloid, through the acinar cells, into the lymphatics or blood stream. This process is accelerated by TSH. It has been estimated that about 0.33 mg. of thyroxine is secreted daily under physiological conditions.

Circulating Thyroid Hormone. The plasma normally contains iodine in the form of inorganic iodide (small amounts, < 2 μg. %, except after administration), thyroxine (6–12 μg. %), and perhaps one of its unidentified metabolites. The thyroxine, which is apparently the circulating form of the hormone, is loosely bound to plasma proteins, and its constituent iodine is referred to and determined as protein-bound iodine (PBI), ranging normally from 4 to 8 μg. per 100 mg. plasma.

It is present in highest concentration in the α- and β-globulin fractions, in lowest concentration in the γ-globulin fraction, the largest amount (about 75%) being bound to albumin. It is believed that, in the tissues, the thyroxine is freed from its protein carrier, passes through the capillary wall and either penetrates the cell wall or exerts its effect upon the cell surface.

Fate of Thyroxine. Thyroxine leaves the circulating plasma rapidly in the tissues. After injecting a large amount (dog), only 50 per cent remains in the blood stream after 3 minutes, and less than 10 per cent after 24 hours. With physiological doses, a small amount (5%) is excreted as such by the liver, in the bile, and by the mucosal cells of the duodenum. There is some evidence of an enterohepatic circulation. The bulk of the thyroxine is degraded, perhaps largely in the liver, with liberation of inorganic iodide. After large doses of thyroxine, a larger proportion is excreted, unchanged, in the bile and intestinal secretions, and ultimately in the feces. Only very small amounts of organically bound I are excreted in the urine. Its partial transformation to a more active compound, triiodothyronine, has been referred to elsewhere (p. 266).

ANTITHYROID AGENTS

A number of chemical agents can interfere with the formation of thyroid hormone by affecting different phases of the mechanism involved in its synthesis. These may be classified conveniently as follows: (1) Thyroid hormone; (2) iodine; (3) thiocyanate; (4) antithyroid agents.

Thyroid Hormone. Administration of thyroid hormone results in regressive changes in the thyroid gland and diminished formation of hormone. This may be due in part to suppression of thyrotrophin (TSH) secretion by the pituitary; however, thyroid hormone apparently also inhibits the action of TSH upon the thyroid gland.

Iodine. Deficiency in iodine intake leads to deficient synthesis of thyroid hormone and compensatory hyperplasia of the thyroid gland (iodine-deficiency goiter). Conversely, administration of iodine to subjects with hyperthyroidism is followed by regressive changes in the gland and diminished output of hormone. High levels of circulating iodide also have a less pronounced and temporary inhibitory effect on thyroid hormone synthesis by the normal thyroid. The mechanism is not clear.

Thiocyanate. Administration of thiocyanate results in enlargement of the thyroid (goiter), if the blood iodide concentration is low, and decreased synthesis of thyroid hormone. This effect is due to inhibition of the uptake, concentration and accumulation of iodine by the thyroid, the gland being obliged to form hormone from the iodine which enters by passive diffusion. If the level of circulating iodide is elevated sufficiently by administration of iodine, enough may enter the gland to permit adequate function, inasmuch as thiocyanate does not influence the synthesizing mechanism per se (i.e., oxidation, iodination of tyrosine and coupling). The mechanism of its action is unknown.

Antithyroid Agents. This designation is applied to a large number of substances which inhibit synthesis of thyroid hormone, apparently by interfering in some way with the oxidation of inorganic iodide, necessary for iodination of tyrosine.

These compounds fall into two catagories: (*a*) those containing a thiocarbamide grouping (e.g., thiourea, thiouracil, and related compounds); (*b*) those containing an aminobenzene grouping (e.g., sulfonamides).

Administration of these antithyroid compounds leads to hyperplasia of the thyroid (goitrogenic action) as a result of excessive stimulation by TSH, secretion of which by the pituitary is no longer adequately restrained by circulating thyroid hormone.

The mechanism of action of these agents is uncertain. The thiourea-like compounds react rapidly with iodine, reducing free iodine to iodide at neutral pH, a phenomenon which could effectively interfere with iodination of tyrosyl groups. However, other substances which react similarly with iodine, such as glutathione, cysteine, and thioglycolic acid, do not inhibit thyroid function. Moreover, the aminobenzene derivatives, which apparently inhibit thyroid hormone formation at this same stage, react only very slowly with iodine. Other possible mechanisms include (*a*) inhibition of the oxidizing enzyme system, (*b*) competition for hydrogen peroxide, or (*c*) competition as a substrate.

ABNORMAL EXCRETION OF IODINE

The increased level of blood iodine in hyperthyroidism (p. 270) is accompanied by a corresponding increase in its excretion by the kidneys, liver, intestine and skin. The daily urine iodine may reach 40 to 950 gamma, depending upon the intake and the concentration in the blood. In some cases a negative balance of as much as 200 gamma daily has been observed. Under such circumstances, the body reserve of 20 to 50 mg. would be exhausted in a short time, but it is probable that the blood iodine concentration and its excretion decrease before this occurs.

Increased excretion of iodine in the urine has been observed during menstruation and pregnancy, in certain acute infections (also in perspiration), during periods of excitement, immediately following partial thyroidectomy in some cases of hyperthyroidism, and after a variety of surgical procedures. Whether or not this phenomenon is dependent upon increased thyroid activity is debatable.

In myxedema, the urine iodine usually decreases more markedly in the fasting state than in normal individuals. Under ordinary conditions of normal iodine intake, little if any deviation from the normal excretion can be observed, since this depends largely upon the supply. However, it has been stated that after ingestion of iodine, the increase in urinary excretion in myxedema is greater and more prolonged than normal. This is perhaps related to the inability of the atrophic thyroid to take up iodine as completely as the normal gland.

Measurement of urinary excretion of I^{131} following administration of tracer doses is employed widely in the study of thyroid function. This subject is discussed in detail elsewhere (p. 271).

ABNORMAL BLOOD IODINE

Increase in blood iodine has been reported in about 40 per cent of cases of lymphatic leukemia and occasionally also in myeloid leukemia. This increase is not related to the basal metabolic rate. Elevated total iodine values have been observed in obstructive and hepatocellular jaundice, and in a variety of other conditions, but the increase is virtually exclusively in the inorganic fraction and is therefore of little or no clinical significance.

Inasmuch as the "protein-bound iodine" (PBI) in the plasma probably represents circulating thyroid hormone, determination of this factor is one of the most direct approaches to the evaluation of the state of thyroid function. Interpretation of abnormalities in PBI are based on the premise that an increase reflects hypersecretion of thyroid hormone and a decrease, hyposecretion. However, the possibility exists that alterations in plasma PBI may at times be due, in part at least, to changes in the rate of peripheral utilization or destruction of the hormone. Although this possibility has not been definitely excluded, the assumption referred to above is usually valid in clinical states.

Values above 8 μg./100 cc. are obtained in hyperthyroidism, the increase roughly paralleling that in BMR. Little or no significant rise may be demonstrable in borderline cases. However, this determination may be useful in indicating the non-thyroid basis for BMR elevations due to other causes and in suggesting the presence of hyperthyroidism at BMR levels within the normal range, e.g., ± 15.

In hypothyroidism, the plasma PBI is low or absent. Following administration of iodide, the blood level of inorganic iodine remains elevated for a longer time than in normal subjects because of lack of "trapping" by the thyroid gland (iodine tolerance test). Low values are obtained also in hypopituitarism.

High levels of PBI have been found in normal pregnancy, associated with elevation of BMR, but unaccompanied by manifestations of thyrotoxicosis. There is at present no adequate explanation for this phenomenon.

It must be remembered that results obtained with this valuable method of study, as with other laboratory procedures, must be evaluated critically. Determination of PBI requires specialized technical skill, care, and experience, and does not lend itself, at the present time, to routine use in general clinical laboratories.

One of the most important sources of error is in the presence of large amounts of nonhormonal iodine. It appears that when the concentration of iodide is high, some will be incorporated in the protein precipitate and be measured as "protein-bound" iodine. The error is even greater in the presence of appreciable amounts of organic iodine compounds, such as are used for bronchography, encephalomyelography, cholecystography, and urography.

Spuriously low values for PBI following administration of mercurial diuretics are attributable to the formation of insoluble mercuric iodate or iodide during the technical procedure. This interference usually lasts only about twenty-four hours but may persist longer in cases in which excretion of the diuretic is delayed. Obviously, medication with antithyroid agents will lower the level of PBI, especially in hyperthyroid subjects. Low values have been obtained in the presence of hypoproteinemia, e.g., the nephrotic syndrome, and also in Addison's disease and infectious diseases.

Elevated levels may be found at times in euthyroid individuals following administration of large amounts of radioactive iodine, such as are used in the treatment of thyroid cancer. This is believed to be due to liberation of excessive amounts of hormone from the thyroid as a result of the heavy radiation.

RADIOIODINE STUDIES

Radioactive iodine (I^{131}) has contributed greatly to present understanding of the mechanism of synthesis of thyroid hormone and of thyroid physiology and iodine metabolism in general. This agent is useful also in the clinical evaluation of the state of thyroid function. Several test procedures in this connection have been devised, a few of which merit consideration here because of their applicability in routine clinical studies. Before discussing these, certain points should be reviewed that have general pertinence to the use of radioiodine for diagnostic purposes.

It is essential to make sure that the patient has received no medication that will influence iodine uptake by the thyroid. Ingestion of iodide causes decreased uptake, especially in thyrotoxicosis, but also to a lesser degree in other conditions. The duration of this effect after cessation of therapy has not been established precisely. It may perhaps last as long as two months. Similar inhibition may persist for six to nine months following administration of iodine-containing radiopaque material for urography, bronchography, cholecystography, and encephalomyelography.

Antithyroid drugs (e.g., propylthiouracil, Tapazol, etc.) inhibit the transformation of inorganic iodide to molecular iodine (p. 267). The iodide taken up by the thyroid in subjects treated with these agents is therefore not retained and incorporated into the organic moiety. Employing diagnostic tracer technics under such circumstances, the thyroid uptake (incorporation) values will be diminished. The duration of this inhibition after cessation of treatment probably depends largely on the duration of therapy. It is generally believed that even after prolonged administration of propylthiouracil the thyroid block will be released two weeks after therapy has been discontinued. In certain experimental animals the I^{131} uptake rises above pretreatment levels during the period of release from the action of antithyroid drugs ("rebound phenomenon"); whether this occurs in man is not certain.

Thiocyanate blocks uptake of iodide by the thyroid. This effect wanes rapidly after discontinuing medication, and is lost within forty-eight hours. Treatment with thyroid hormone preparations suppresses thyroid activity, probably through inhibition of thyrotrophin secretion from the anterior pituitary. This inhibition of thyroid function, including uptake of I^{131}, reverts to normal gradually after discontinuing medication. It probably takes about two months for the effect to disappear completely.

Uptake of I^{131} by the thyroid is suppressed by administration of ACTH or cortisone. In subjects with congestive heart failure and edema, appreciable amounts of I^{131} accumulate in the extrathyroid tissues following administration of a tracer dose. Both thyroid uptake and urinary excretion of I^{131} may be decreased in such cases.

Because iodide passes the placental barrier, and is taken up by the fetal thyroid after the eighteenth week of gestation, radioiodine should not be given to pregnant women after this period. It is probably inadvisable to use it even in earlier stages of pregnancy. Moreover, it should be remembered that the cumulative radiation effect of repeated tracer doses may be potentially harmful.

Thyroid Uptake of I^{131}. The radioiodine, as potassium or sodium iodide, is given by mouth in aqueous solution. Up to an amount of about 150 microcuries (μc.), the percentage uptake by the thyroid appears to be independent of the dose, which is determined chiefly by the type of measuring equipment available. With the usual counting devices the uptake can be determined satisfactorily after doses of 20 to 50 μc. With the use of scintillation counters, smaller amounts can be detected; uptake studies have been reported following administration of as little as 1 to 5 μc., but 5 to 10 μc. are commonly employed.

Measurements of radioactivity are made over the thyroid gland by means of a Geiger tube, usually twenty-four hours after administration of the tracer dose. In normal (i.e., euthyroid) subjects the activity over the thyroid (i.e., uptake) at that time is 10 to 40 per cent of the quantity administered. Abnormally high values are obtained in hyperthyroidism and subnormal values in hypothyroidism.

One of the suggested variations of this procedure involves intravenous injection of the I^{131} (20–100 μc.), and thyroid uptake measurement one hour later. Normal values range from 4 to 11 per cent of the administered dose. It is stated that this procedure permits more accurate distinction between the euthyroid and the hyperthyroid state than do twenty-four hour measurements.

Urinary Excretion of I^{131}. Inasmuch as the iodine which is not trapped by the thyroid gland is largely excreted in the urine, the amount of radioiodine excreted in the urine after administration of the test dose reflects the amount taken up by the thyroid. Measurements are commonly made over 24 or 48 hour periods, normal values ranging from 60 to 90 per cent of the administered dose. Subnormal urinary excretion indicates increased uptake by the thyroid, i.e., hyperthyroidism. The majority of thyrotoxic subjects excrete less than 40 per cent. Abnormally high excretion (over 90 per cent) occurs in hypothyroidism.

Some feel that measurement of excretion during the interval between 12 and 24 hours is more valuable in the diagnosis of hyperthyroidism than 48 hour measurements. There are indications that 72 or 96 hour measurements are more valuable than 48 hour determinations in the diagnosis of hypothyroidism.

Decreased excretion may occur in euthyroid subjects with impaired renal function, erroneously suggesting hyperthyroidism. One distinct disadvantage is the fact that the cooperation of the patient is usually required in the complete collection of urine for a rather long period. Incomplete collection may lead to a falsely positive diagnosis of hyperthyroidism and a falsely negative diagnosis in hypothyroidism.

Conversion Ratio of I^{131}. Studies of thyroid uptake of radioiodine, as outlined above, measure two functions of this gland: (1) its capacity for accumulating iodide, and (2) its capacity for retaining organically bound iodine. By determining the radioactive protein-bound iodine in the blood plasma twenty-four hours following administration of a tracer dose, an insight is obtained into the mechanism of release of hormone from the gland. The "conversion ratio," expressed as $\frac{\text{Plasma protein-bound } I^{131}}{\text{Total plasma } I^{131}} \times 100$, is an index of thyroid hormone release into the circulation.

Twenty-four hours after oral administration of 100 μc. of I^{131}, blood is drawn and the plasma (or serum) separated. The total I^{131} content of a plasma sample is determined (i.e., radioactivity). Another sample is deproteinized and the I^{131} content (radioactivity) of the protein precipitate is measured. In euthyroid subjects the conversion ratio is 15 to 40 per cent. It is increased (50–100%) in hyperthyroidism and decreased (0–5%) in hypothyroidism.

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CHAPTER XIII

Sodium, Potassium and Chloride Metabolism

The metabolism of these substances is intimately related to the water balance and acid-base equilibrium of the body, which are considered in detail elsewhere (pp. 295, 323) and which will therefore be mentioned here only briefly. Sodium, potassium, and chloride are concerned in the following fundamental physiologic processes: (1) the maintenance of normal water balance and distribution; (2) the maintenance of normal osmotic equilibrium; (3) the maintenance of normal acid-base equilibrium (physiologic neutrality); (4) the maintenance of normal neuromuscular irritability. The maintenance of normal hydration and osmotic pressure depends primarily upon the total base content of the body fluids (p. 301). Since sodium constitutes the largest fraction of the total base of the extracellular fluids $\left(\frac{142}{155}\right)$, it plays a dominant role in this connection (p. 311). At any given H_2CO_3 concentration, the hydrogen ion concentration of plasma and other extracellular fluids is dependent upon the concentration of bicarbonate $\left(H^+ = k\,\frac{H_2CO_3}{BHCO_3}\right)$. Since the bicarbonate content depends upon the amount of total base present in excess of anions other than HCO_3, and since Cl constitutes by far the largest fraction of the total anions of the plasma $\left(\frac{103}{155}\right)$, it naturally follows that the maintenance of the normal pH depends largely upon the presence of normal concentrations of sodium and chloride (p. 311). The importance of a proper balance between Na, K, Mg and Ca in the maintenance of normal neuromuscular irritability and excitability is well known. It may be expressed as follows:

$$\text{Irritability} \propto \frac{[Na^+] + [K^+]}{[Ca^{++}] + [Mg^{++}] + [H^+]}$$

Potassium is the chief cation of the muscles and of most other cells (intracellular fluid), whereas sodium is the chief cation of extracellular fluids of the body. Some movement of K occurs from cells to extracellular fluids (including plasma), especially under abnormal conditions of excessive loss of intracellular fluid, the mobilized K being usually excreted promptly in the urine. Some movement of Na occurs in the opposite direction, i.e., from extra- to intracellular fluid, under these conditions. Any considerable increase or decrease in K concentration in the extracellular fluids produces serious and at times fatal disturbances in muscle irritability, respiration and myocardial function. Moreover, no other cation can entirely replace potassium in the intracellular fluid without interfering to a certain extent with the functional activity of the cell. In low concentrations potassium is excitatory and in higher concentrations it is inhibitory, these effects being particularly important in relation to nerve synapses or myoneural junctions. Under normal conditions, its effects resemble those of parasympathetic stimulation, and are usually inhibited by calcium (e.g., neuromuscular excitability). The K/Na ratio is also important in this connection. Potassium and calcium modify the most fundamental properties of protoplasm and cells, including the permeability of cell membranes, and thus they play a role in almost all "vital" processes.

ABSORPTION AND EXCRETION

An average adequate diet contains about 3 to 4 gm. (75–100 mEq.) of K and 8 to 15 gm. (130–250 mEq.) of NaCl daily, the latter being contributed to by the addition of salt to the food. Normally, Na, K, and Cl are practically completely absorbed from the gastrointestinal tract, less than 2 per cent of ingested Na and less than 10 per cent of the K being eliminated in the feces. In subjects with diarrhea, large amounts are lost in the feces, owing in large measure to failure of reabsorption of constituents of digestive fluids, the electrolyte composition of which is indicated in Figure 31 (p. 313) and Table 11. Na and Cl are eliminated chiefly in the urine and, to a lesser extent, in the perspiration. K is normally eliminated almost entirely in the urine.

Excretion in Urine. The amounts excreted in the urine are indicated elsewhere (p. 578). Suffice it to state here that under normal conditions the daily urinary excretion of these electrolytes approximates the intake except under conditions of sudden increase of NaCl intake following a period of restriction. Na and Cl are removed from the circulating plasma by glomerular filtration, over 1000 gm. of NaCl entering the renal tubules daily in the glomerular filtrate. Under normal conditions 99 per cent or more undergoes reabsorption into the blood stream by the tubular epithelium. About 80 to 85 per cent of this is reabsorbed in the proximal portion of the tubule, the remainder in the distal portion. Adrenocortical hormones probably influence chiefly the latter fraction (enhancing reabsorption of Na and Cl) but perhaps also the former. In the distal portion, too, about 1 to 2 per cent of the Na^+ reabsorption occurs in exchange for H^+ ions (also for K^+) (p. 330) as part of the mechanism of acidification of the urine. Under normal conditions the regulation of Na and Cl reabsorption and conservation is so efficient that equilibrium can be maintained on a NaCl intake as low as 0.5 gm. daily, Na and Cl virtually disappearing from the urine.

Na and Cl are substances designated as "threshold bodies" which, being of value to the organism, are, after their passage through the glomeruli, reabsorbed into the circulation in order to maintain their normal concentration in the blood. The normal "renal threshold" value for sodium chloride is 560 to 570 mg. per 100 cc. of blood plasma. In the normal individual, as the plasma chloride concentration descends to this level, the elimination of chloride in the urine decreases and finally ceases entirely. Under normal conditions the quantity of chloride eliminated in the urine approximates the chloride intake; in cases in which there is marked variation in the quantity ingested it may require some time for an equilibrium to be established. For example, if the normal individual is kept upon an adequate sodium chloride intake (8–15 gm.), the sudden addition of a large quantity of salt is usually followed by complete elimination of the excess within forty-eight hours. However, if the individual has been maintained for prolonged periods of time on a low salt intake, the elimination of salt administered subsequently in large quantity may be delayed for several days, the body retaining the added salt with greater tenacity. In conditions in which the elimination of chloride through other channels such as the skin (excessive perspiration) or gastrointestinal tract (vomiting or diarrhea) is increased, the urine chloride content is correspondingly diminished.

K is removed from the plasma by glomerular filtration and, like Na and Cl, undergoes extensive (over 90%) reabsorption in the tubules. However, unlike Na and Cl, it is also excreted into the tubular lumen by the cells of the distal portion of the renal tubules. This active process, which apparently competes with H^+ ions in the Na^+ exchange mechanism (p. 330), is perhaps the phase of K excretion which is influenced by adrenocortical hormones (enhancing K excretion). In the presence of normal kidney function, K is very promptly and efficiently removed from the blood plasma even in the face of a considerably increased supply, preventing undue increase in its concentration in the extracellular fluids.

Table 11. Approximate Concentration of Certain Electrolytes in Digestive Fluids and Perspiration (mEq./liter)

FLUIDS	NA	K	CL	HCO_3
Gastric juice	20	8	145	0
Pancreatic juice	140	5	40	110
Jejunal juice	138	5	110	30
Bile	140	8	108	38
Perspiration	82	5	85	0
Blood serum	140	4.3	100	25
Interstitial fluid	145	3.3	110	28

Excretion by the Skin. The Na and Cl content of perspiration is usually lower than that of blood plasma (Table 11). Their concentrations in sweat are decreased by the salt-active adrenal cortical hormones (p. 432), which are secreted in increased amount during acclimatization to heat. This constitutes an adaptive mechanism for conservation of body Na and Cl, the sweat glands producing a progressively more dilute fluid. In normal subjects, the loss of Na and Cl in the perspiration is compensated by a corresponding diminution in their urinary excretion. However, considerable amounts may be lost from the body under conditions of prolonged strenuous exertion at extremely high temperatures and humidity (p. 284).

Excretion in Digestive Fluids. The approximate electrolyte composition of certain digestive fluids is indicated in Figure 31 (p. 313) and in Table 11. Under normal conditions these fluids are virtually completely reabsorbed in the bowel, but, in the presence of protracted vomiting or diarrhea, it is obvious that large amounts of water and electrolytes may be lost from the body. This is of great importance in the treatment of the dehydration and dislocations of acid-base balance that occur under these circumstances.

The chloride content of the gastric juice of normal individuals may vary considerably but is relatively constant for each individual; this individual variation, however, is in no case so great as would appear to be indicated by the rather wide variation in free acid secretion. Relatively normal total chloride values may frequently be observed in cases exhibiting extremely low or absent free HCl in the gastric juice (false achlorhydria), a fact which is due usually to excessive neutralization by regurgitation of alkaline material from the duodenum.

NORMAL BLOOD NA, CL, AND K

The normal amounts of Na, Cl, and K in 100 ml. of blood plasma (or serum) are as follows: Na, 300–335 (average 320) mg. (132–144 mEq./liter); Cl, 340–370 mg. (96–105 mEq./liter), or 570–620 mg. expressed as NaCl; K, 14–19 (average 16) mg. (3.6–4.8 mEq./liter). In man, erythrocytes contain little or no Na, smaller amounts of Cl than does the plasma, and large amounts of K (about 370 mg./100 grams). The distribution of Cl^- between plasma and erythrocytes is similar and is intimately related to that of HCO_3^-, in accordance with Donnan's law governing the distribution of diffusible monovalent ions (p. 305). The factors which influence this distribution and the changes that occur under physiological conditions are considered elsewhere (p. 306). Suffice it to recall here that shifts of Cl^- and HCO_3^- between the plasma and the erythrocytes play an important role in the maintenance of a constant pH in the blood.

Because of the unequal distribution of these elements between the blood plasma and erythrocytes, they should always be determined in the plasma (or serum), not in whole blood. In the case of K, care must be exercised to avoid hemolysis, which liberates K into the plasma from the hemolyzed cells. Obviously, the Cl content of whole blood varies considerably with variations in the number of red cells, being relatively high in anemia (i.e., larger proportion of Cl-rich plasma) and relatively low in polycythemia. Moreover, because of the shift of Cl between the plasma and red cells that occurs with changes in CO_2 tension (p. 326), blood drawn for plasma Cl determinations should be collected under anaerobic conditions, for, if exposed to air, CO_2 escapes from the blood, the plasma Cl concentration being thereby increased.

The concentrations of Na, Cl, and K in the plasma vary but slightly under physiological conditions. The plasma Cl may fall somewhat during periods of active gastric secretion of HCl and may rise again in the post-digestive period (reabsorption from intestine). It is affected but slightly by marked variations in Cl intake; the same is true of the plasma Na and K concentrations. The plasma K decreases somewhat during periods of increased carbohydrate utilization (oxidation and glycogen deposition), as following administration of glucose or insulin.

This is due apparently to its passage from extracellular fluids into the cells in increased amount under these circumstances (p. 32).

The influence of adrenal cortical hormones (especially the desoxycorticosterones) in increasing plasma Na and Cl and decreasing K is considered elsewhere (p. 432). It is the result apparently of their effect on renal tubular reabsorption of these ions and on their distribution between intra- and extracellular fluids.

DISTRIBUTION AND INTERMEDIARY METABOLISM

Reference is made elsewhere (p. 303) to differences in the composition of intra- and extracellular fluids. These are illustrated in Figure 29 (p. 304). We are concerned here mainly with the striking differences in the concentrations of Na, K, and Cl in these fluid compartments. It should be understood that the concentration of electrolytes varies in the intracellular fluid of individual tissues, but the general pattern is rather uniform. Skeletal muscle is the tissue studied most thoroughly in this connection. The rather strict localization of relatively large amounts of K in the intracellular and of Na and Cl in the extracellular fluid is attributable to a rather high degree of selective relative impermeability of most cell membranes to these ions. However, this impermeability is neither absolute nor fixed. In different functional states and under certain abnormal circumstances the composition of the intracellular fluid may be altered as a result of loss of K from and entrance of Na into the cells. These changes (i.e., abnormal) are often accompanied by alterations in the concentrations of Cl^- and HCO_3^- in the extracellular fluid, which may seriously affect the state of "acid-base" balance (p. 282).

The potassium within the cells is only partly ionized and osmotically active, some being bound to protein and some to phosphate and other complexes. As cell protein undergoes degradation, K is released to the extracellular fluid in a rather fixed proportion to the N mobilized (2.7 mEq. K (110 mg.) per gram N). Under normal conditions, this catabolic process is balanced by the entrance into the cell of an equivalent amount of K as a part of the process of protein anabolism.

Potassium moves into the cells at an accelerated rate during periods of increased glucose utilization (glycogenesis, glycolysis, oxidation), e.g., after administration of glucose or insulin. This may be related to the increase in phosphorylative processes that occurs under these circumstances, and may be reflected in a temporary fall in plasma K concentration. Deposition of glycogen (liver and muscles) requires an increment in intracellular fluid, about 1 cc. per gram, involving the entrance into the cell of about 6 mg. (0.15 mEq.) of K per gram. Breakdown of glycogen is accompanied by passage of an equivalent amount of K in the opposite direction. Accordingly, potassium leaves muscle cells at an increased rate during strenuous exercise, and reenters more rapidly during periods of rest.

Some sort of equilibrium is maintained normally between the high cellular (150 mEq./liter) and low extracellular (5 mEq./liter) concentrations of K, and the reverse situation with regard to Na, although the factors operating are not well understood. The adrenal cortical hormones and the kidneys are probably involved, as may be also the oxidative energy of the cell. Under normal conditions, Na and K entering the body are distributed rapidly throughout the extracellular fluids, an equivalent amount being excreted within a short time, almost entirely in the urine. Very little Na is transferred to the cells; studies with radioactive K indicate that its transfer through the cell membrane is slow as compared to that of water, the labeled K attaining equilibrium with the intracellular K only after fifteen hours, whereas deuterium-labeled water attains equilibrium with the total body water in two hours. The addition of Na to the extracellular fluids is followed by a temporary shift of water to that compartment from the cells, osmotic equilibrium being thus preserved. The addition of K, on the other hand, is followed promptly by increased renal excretion of K (diuresis), preventing its accumulation in excess in the extracellular fluids. Certain of the factors which influence renal excretion of K are discussed elsewhere, viz., adrenocortical hormones (pp. 290, 432), K concentration in the cells (p. 330), and the state of acid-base balance (p. 331).

Table 12. Approximate Total Amount of Na, K, Cl, and HCO_3 in Body Fluid Compartments of a 70-Kg. Man (in Milliequivalents)

FLUID	NA	K	CL	HCO_3	H_2O
Extracellular	2000	45	1500	400	14,000 ml.
Intracellular	400	5200	110	340	35,000 ml.

ABNORMAL NA, CL, AND K METABOLISM

The relation of alterations in the metabolism of Na, Cl, and K to disturbances of water and acid-base balance is discussed elsewhere (pp. 312 ff., 333 ff.), and will be referred to here only briefly. The present discussion is concerned mainly with the causes of clinical abnormalities in these elements. It is advisable to consider them together because primary change in one is almost invariably accompanied or followed by change in one or both of the others.

Routes of Loss of Na, Cl, and K. Excessive amounts of various electrolytes may be lost from the body by way of the gastrointestinal tract (vomiting, diarrhea, fistulas, tube drainage), kidneys (diuresis), or skin (excessive sweating). Electrolyte depletion may occur also as a result of rigid restriction of electrolyte intake (e.g., potassium, in starvation).

Gastrointestinal. Normally, all but about 100 to 200 cc. of the large amounts of water and electrolytes (Table 14, p. 296) entering the digestive tract are reabsorbed in the bowel. Under abnormal conditions, however, enormous quantities of these digestive secretions may be lost from the body, viz., vomiting, diarrhea, tube drainage, fistulas. The possible magnitude of these losses is indicated in Table 13. These figures may be exceeded in certain diseases because the volumes of gastric and intestinal secretions may be increased due to local irritation, e.g., inflammation. On the other hand, the concentration of electrolytes in certain of these fluids may fall during protracted periods of loss (e.g., ileostomy), perhaps in part due to a process of adaptation, and in part reflecting their diminishing concentrations in the plasma.

Under these circumstances, which are encountered frequently clinically, over 8000 cc. of water, 900 mEq. Na, 820 mEq. Cl, and 150 mEq. K may be lost from the body in twenty-four hours. The gravity of such situations becomes apparent when these values are compared with the total body content of these substances (Table 12, p. 279). Obviously, losses even remotely approaching these magnitudes cannot be tolerated for longer than very brief periods, emphasizing the importance of prompt and active treatment of clinical states of dehydration.

Table 13. Volume and Electrolyte Composition of Body Fluids Commonly Involved in Production of Dehydration

(Figures in parentheses are average values.)

	H_2O cc.	mEq. per liter			Total possible loss (mEq.)		
		Na	Cl	K	Na	Cl	K
Gastric juice (resting)	2500	10–120 (60)	7–160 (85)	0.5–35 (9)	300	400	85
Pancreatic juice	1000	110–150 (140)	20–80 (40)	2–8 (5)	150	80	8
Small bowel	3000	80–150 (135)	40–140 (105)	2–10 (5)	450	420	30
Saliva	1500	(9)	(10)	(25)	15	15	40
Sweat	14,000	40–80	35–70	3–5	110	100	70

Skin. Under conditions of ordinary moderate environmental temperature and humidity, there is little or no active sweating. The water loss by "insensible perspiration" (vaporization from skin and lungs) is approximately 500 cc./sq. meter body surface area (p. 295), and no electrolytes are lost by this route. The volume of "insensible perspiration" is increased in fever, roughly in proportion to the temperature elevation.

The sweating mechanism is invoked by increased atmospheric temperatures and humidity and by physical exertion. Heavy work in hot and humid environments, e.g., steel mills, deep mines, etc., may involve enormous losses of sweat, as much as 2000 cc. per hour. The composition of perspiration varies considerably. Its electrolyte content (40–85 mEq. base/liter) is much lower than that of plasma (155 mEq. base/liter) and, therefore, prolonged, profuse sweating results in loss from the body fluids of relatively more water than Na and Cl, with consequent hypernatremia and hyperchloremia (p. 287).

The figures in Table 13 (p. 280) represent theoretically possible losses, but these seldom occur under clinical conditions because of the operation of an adaptation mechanism, apparently involving increased secretion of salt-active adrenal cortical hormones. The process of acclimatization to high temperatures and humidity is accompanied by a decrease in the concentrations of Na and Cl in the sweat, e.g., to 2 to 5 mEq./liter.

URINE. Under normal conditions the urine is the route of elimination of surplus water and electrolytes (and other solids). In the presence of normal kidney function, and in the absence of forces operating directly on renal excretory mechanisms (diuretic drugs, hormones, kidney disease), the quantities excreted by this route therefore vary inversely with the amounts lost through other channels, e.g., by vomiting, diarrhea, or excessive sweating. In this manner the kidneys attempt to preserve the body fluid content and composition in the face of abnormal losses by other avenues of elimination.

Progressive dehydration, with electrolyte depletion, may occur during prolonged periods of diuresis, as in diabetes mellitus, chronic glomerulonephritis, and Addison's disease, and as a result of administration of potent diuretic agents, e.g., mercurial diuretics. The nature of the underlying mechanism varies in different conditions, and is reviewed in discussions of other aspects of these disorders (q.v.). The most important underlying feature common to most of them is diminished renal tubular reabsorption of Na (also H_2O, and usually Cl) from the glomerular filtrate. As indicated elsewhere (p. 290), excessive amounts of K are also lost frequently by this route.

In diabetes insipidus, enormous quantities of water (up to 30 or more liters) may be excreted by the kidneys, without comparable losses of electrolytes (i.e., very low urine specific gravity). If adequate amounts of water are not taken in, this type of dehydration may be accompanied by hypernatremia and hyperchloremia.

General Principles in Electrolyte Abnormalities. No uniformly applicable rules can be set down for the behavior of electrolytes in various disease states because of the wide range of individual variability in several important modifying factors, e.g., the acidity of the gastric juice (in vomiting), the duration and severity of the abnormality, the state of renal function, and the presence of other complications. However, certain principles may be outlined, which serve to indicate the general trend of abnormalities in Na, Cl, and K under commonly encountered clinical conditions. It must be understood that these generalizations are subject to modification in individual instances.

1. Na and Cl are lost predominantly from extracellular fluids, K almost exclusively from intracellular fluids.

2. Although, in dehydration, fluid is lost primarily from the extracellular compartment (early, mainly interstitial fluid), this loss is shared also by the intracellular fluids almost from the start, although to a lesser extent. For practical purposes, therefore, it may be assumed that in dehydration of more than slight degree, due to excessive loss of body fluids (vomiting, diarrhea, diuresis, sweating), the body has lost water, Na, Cl, and K, as well as smaller amounts of other substances (e.g., PO_4, SO_4).

3. Under these conditions (i.e., vomiting, diarrhea, diuresis), passage of K out of the cells into the extracellular fluid is accompanied by passage of somewhat smaller amounts of Na in the opposite direction. As much as one-half of the intracellular K can be replaced by two-thirds the equivalent amount of Na under these circumstances.

In the presence of normal renal function, the K is excreted in the urine (in addition to the primary route of excessive fluid loss, e.g., feces, vomitus) with an excess of anion, chiefly Cl, drawn from the extracellular fluid compartment. This extracellular Cl^- deficit is balanced by a simultaneous increase in HCO_3^-.

K moves from extracellular into intracellular fluids when an adequate exogenous supply is provided, especially in states of K-depletion.

4. The above-mentioned interrelations may be expressed as follows: (a) The intracellular Na tends to increase as the intracellular K decreases. (b) The plasma (extracellular fluid) Cl tends to vary directly, and the plasma HCO_3 indirectly as the intracellular K. As indicated above, these interrelationships are dependent upon the presence of adequate kidney function, i.e., adequate urinary excretion of the mobilized K with an excess of Cl. In many cases of dehydration these changes are not observed because of the complicating factor of renal functional impairment. They may manifest themselves, however, as renal function improves during periods of rehydration by NaCl solutions (see below).

5. Under the conditions mentioned above, i.e., K-depletion and adequate kidney function, administration of Na, without K (e.g., NaCl solution), results in entrance of additional Na into the cells and passage of additional K from the cells into the extracellular fluid. This aggravates the K depletion and also the associated changes in plasma Cl and HCO_3.

6. Excessive loss of K, which is ultimately derived from intracellular fluids, results, therefore, in a tendency toward a hypochloremic type of alkalosis in subjects with adequate kidney function. Consequently, the alkalosis due to primary excessive loss of Cl (vomiting in duodenal ulcer) will be aggravated by this mechanism. Primary K deficit due to adrenocortical hyperfunction (Cushing's syndrome) also results in alkalosis of this type. Treatment of such conditions with K-free solutions (NaCl and NH_4Cl), although capable of restoring the extracellular fluid volume fairly satisfactorily, cannot completely correct the alkalosis in severe cases because of the persistent and increasing K deficit. Similarly, administration of NaCl solutions (and Na lactate or bicarbonate) to subjects with acidosis due to primary excessive Na loss, e.g., in diarrhea, although capable of replenishing the extracellular fluids and correcting the acidosis, may result in alkalosis and progressively increasing K deficiency.

7. Uncomplicated acidosis (e.g., Na loss in diarrhea or diabetes mellitus; NH_4Cl administration) is accompanied by a tendency toward decrease in Na and increase in K in both cells (muscle) and plasma. In uncomplicated alkalosis (e.g., vomiting or $NaHCO_3$ administration), the tendency is toward a decrease in cell and plasma K and an increase in intracellular Na.

8. In the face of losses of H_2O and electrolytes, homeostatic mechanisms, mainly renal, operate to prevent or to minimize changes in composition of the

extracellular fluids. Thus, unless the disturbance is marked, and the homeostatic mechanisms are overwhelmed or renal function is impaired, excess K entering the plasma from the cells is excreted promptly in the urine, preventing undue rise in plasma K concentration. Similarly, excessive loss of Na tends to be balanced by loss of equivalent amounts of H_2O, thus limiting the decrease in plasma Na concentration and osmotic pressure that would otherwise occur.

Obviously, therefore, relatively large amounts of Na, K, and H_2O may be lost from the body without comparable alteration in the concentrations of Na or K in the plasma. The true extent of the deficits can be estimated only by measuring the total extracellular (p. 299) and intracellular (p. 300) fluid volumes in addition to the plasma electrolyte concentrations.

DECREASED PLASMA SODIUM (HYPONATREMIA) AND CHLORIDE (HYPOCHLOREMIA)

Gastrointestinal Disorders. Perhaps the most common clinical causes of dehydration and electrolyte deficit fall into this category. They include: (*a*) prolonged vomiting (e.g., peptic ulcer, pylorospasm, gastric malignancy, gastritis, intestinal obstruction, peritonitis, gallbladder disease, uremia, pregnancy toxemia, etc.); (*b*) diarrhea (e.g., ulcerative colitis, enteritis of various types); (*c*) external fistulas (e.g., biliary, pancreatic, intestinal; ileostomy, cecostomy); (*d*) tube drainage (e.g., pre- and postoperative).

In these, as in other forms of dehydration, if the Na loss is of slight or moderate degree, the concentration of Na in the extracellular fluids, including the blood plasma, may be maintained within normal limits by (*a*) renal conservation (i.e., decreased excretion) of Na, and/or (*b*) renal excretion of an equivalent amount of extra water, thus preserving isotonicity. The same is true of Cl, but to a lesser extent. With continuing losses, this renal defense of the composition of extracellular fluids breaks down, with consequent hyponatremia and hypochloremia. Complicating renal disease or functional impairment in tubular reabsorption processes (p. 284) interfere with these regulatory mechanisms, hastening and aggravating the electrolyte abnormalities.

In vomiting, the nature of the alteration in the plasma electrolyte pattern depends on the character of the fluid lost, i.e., on the location of the lesion and the acidity of the gastric juice. In stenosing duodenal ulcer, for example, the vomitus (highly acid) contains large amounts of Cl and relatively little Na, resulting in hypochloremic alkalosis (p. 341). With continued vomiting, the Na concentration of the gastric juice often rises considerably, at times to about one-half the Cl concentration. In cases of obstruction lower in the small intestine, e.g., ileum, the vomitus may contain relatively highly alkaline pancreatic juice in addition to the gastric juice, usually of considerably lower acidity than in peptic ulcer. Under such circumstances, the Na loss will tend to be greater and the Cl loss smaller than in the latter condition. The same is true usually in gastric carcinoma, in which the acidity of the gastric juice is generally low. In such cases, the loss of Na tends to parallel that of Cl. Whether alkalosis or acidosis occurs is determined by the pH of the fluid lost, i.e., whether it contains relatively more or less Cl (in relation to Na) than the blood plasma (p. 312). As indicated elsewhere (p. 282), the extent of K loss also exerts an influence in this connection, K depletion tending to lower the Cl^- and raise the HCO_3^- in the extracellular fluids (i.e., to increase the pH).

The same principles apply in diarrhea, external fistulas, prolonged tube drainage, etc. As a rule, however, these conditions produce more extensive loss of K (p. 280) than occurs in vomiting, and the alteration in extracellular electrolyte pattern is more consistently in the direction of acidosis. One exception is the rather rare condition of congenital, noninfectious diarrhea in infants, characterized by severe K loss and alkalosis (p. 342).

Diabetes Mellitus. Na and Cl are lost in large amounts during the progress of inadequately treated diabetes, and particularly in the course of development of diabetic acidosis. This is due in part to the incident diuresis (accompanying the glycosuria and acidosis), and in part to vomiting, which is a common feature of diabetic acidosis. The Cl loss in the urine is contributed to also by the depletion of intracellular K (p. 290).

In the presence of acidosis, the plasma Na concentration is almost invariably low, whereas the Cl may be normal, subnormal, or at times even increased, depending largely on the ratio of Cl to water loss and on shifts of these substances between extra- and intracellular fluids. The supervention of renal insufficiency (hyponatremia, dehydration, vascular collapse) often modifies the pattern of electrolyte abnormalities.

Kidney Disease. Hyponatremia and hypochloremia occur frequently in chronic glomerulonephritis with renal failure. In this condition, several factors contribute to these abnormalities: (*a*) loss of Na and Cl by vomiting, diarrhea, and previous polyuria; (*b*) decreased capacity of the kidneys to retain (i.e., reabsorb) Na and Cl in the presence of plasma Na and Cl deficits; (*c*) impaired capacity for acidification of the urine (i.e., $Na^+ : H^+$ exchange, p. 330) and for ammonia formulation (p. 331), with consequent loss of excessive amounts of Na in the urine. The gastric acidity is often low in uremic subjects, increasing the loss of Na in the vomitus.

Depletion of Na and, to a lesser extent, Cl, occurs also in types of renal tubular inadequacy (organic disease or functional) characterized by the syndrome referred to as "renal tubular acidosis," or "tubular insufficiency without glomerular insufficiency" (p. 337). In these conditions ("salt-losing nephritis"), reabsorption of Na and Cl is inadequate. Acidosis and vascular collapse develop, the clinical and electrolyte pictures resembling Addison's disease.

Hyponatremia and hypochloremia are commonly encountered in severe bichloride nephrosis, perhaps due mainly, but not entirely, to loss by excessive vomiting and diarrhea.

The occurrence of these abnormalities in the nephrotic syndrome (with edema) is based on factors which operate to produce similar changes in subjects with congestive heart failure, i.e., restricted NaCl intake and induced diuresis (low salt syndrome, p. 285).

Excessive Sweating. Enormous quantities of sweat (10–14 liters daily) may be lost by individuals working in very hot and humid environments (p. 280). Loss of this hypotonic fluid (p. 313) may result in increase in plasma Na and Cl concentrations (p. 287), particularly since adaptation to these environments is accompanied by decrease in the NaCl concentration of the sweat from the normal 40 to 80 mEq./liter to as low as 2 to 5 mEq./liter. However, if such subjects drink large amounts of water and no provision is made for simultaneous replacement of the lost Na and Cl, the concentration of these elements in the extracellular fluids may fall, with the development of manifestations resembling those of adrenal insufficiency, including renal functional impairment and nitrogen retention in the blood. This phenomenon is the basis for the production of so-called "miners' " or "stokers' " cramp, characterized by severe, localized muscle spasms, brought on particularly by exertion, and often preceded by weakness of the affected muscles.

Excessive sweating has been reported to occur during hot weather in children with fibrocystic disease of the pancreas. The sweat in this condition was found to contain abnormally large amounts of Na (av. 133 mEq./liter) and Cl (av. 106 mEq./liter), the concentrations approximating those in the blood plasma. No renal or adrenocortical abnormality was demonstrable, and a disturbed sweating mechanism has been postulated. Under these circumstances, administration of large amounts of NaCl-poor fluids may result in hyponatremia and hypochloremia.

Infectious Diseases. Hypochloremia has been observed in several acute infections, including pneumococcic pneumonia, rheumatic fever, and meningococcic meningitis. A similar, but less marked decrease may occur in erysipelas, typhoid fever, and tuberculosis. This phenomenon has received particular attention in pneumonia.

The urine Cl is also decreased, as is the serum Na concentration. At the time of crisis in pneumonia, the urine and plasma Cl increase sharply to essentially normal levels. Studies of extracellular fluid volume suggest that hypochloremia and hyponatremia of this type, at least in pneumonia, may be explained on the basis of expansion (i.e., dilution) of the extracellular fluid compartment.

Adrenocortical Insufficiency (Addison's Disease). The characteristic metabolic manifestations of adrenocortical insufficiency are discussed in detail elsewhere (p. 436ff.). The fact should be emphasized that, except in crisis, the serum Na and Cl concentrations (also K) are usually within normal limits. The early abnormalities consist in excessive excretion of Na and Cl (and H_2O) in the urine, usually in such proportions as to preserve the normal electrolyte pattern in the extracellular fluids in the face of progressive dehydration and NaCl depletion. In adrenal crisis, however, there is characteristically a sharp decrease in serum Na and Cl concentrations (and increase in K, p. 438), probably reflecting a break in fundamental regulatory processes, including renal mechanisms.

"Low Salt Syndrome." This designation is applied to a state of hyponatremia and hypochloremia, with other rather characteristic manifestations, that occurs under certain clinical circumstances. Other associated phenomena include: renal failure, with progressive oliguria and increasing blood urea N; progressive decrease in urine Cl excretion; at times, increasing weight (edema). Common symptoms are anorexia, nausea, drowsiness, weakness, lethargy, and abdominal and muscular cramps. In most instances the drop in Na exceeds that in Cl, resulting in acidosis.

In addition to conditions mentioned elsewhere (e.g., "salt-losing nephritis" (p. 284); excessive sweating (p. 285); diarrhea (p. 283), this syndrome is encountered under conditions in which the following combination of circumstances occurs: (*a*) rigid restriction of NaCl intake; (*b*) administration of excessively large amounts of salt-poor fluids (water, glucose solution); (*c*) stimulation of urinary excretion of Na and Cl by mercurial diuretics; (*d*) in the majority of cases there is some degree of renal functional disturbance (e.g., in congestive heart failure) or organic kidney disease.

The clinical disorders in which these circumstances coexist most commonly are: (1) congestive heart failure, treated with low NaCl diets, high water intake, and mercurial diuretics; (2) the nephrotic syndrome, treated similarly; (3) at times in patients with hepatic cirrhosis and ascites, treated in the above manner, with repeated withdrawal of large amounts of ascitic fluid by paracentesis; (4) after operations on dehydrated patients, if large amounts of glucose solution are injected without adequate amounts of NaCl; (5) occasionally in patients with hypertension maintained on very low salt diets.

Adrenocortical Hyperfunction (Cushing's Syndrome). A hypochloremic type of alkalosis occurs rather consistently in this condition. As indicated elsewhere (p. 290, 342), it is due primarily to depletion of intracellular K, which, passing into the extracellular fluid, is excreted in the urine, carrying with it an excess of Cl, which is replaced in the intracellular fluids by HCO_3.

Miscellaneous. Hyponatremia and hypochloremia may occur in the rather rare condition of acute hyperparathyroidism, with hemoconcentration, oliguria, and renal failure. These changes are believed to result from the active diuresis which occurs in such cases.

Hypochloremia, with no change in Na, occurs in bromide intoxication, the latter ion replacing Cl in the body fluids.

Hypochloremia may occur as a result of administration of excessive amounts of absorbable alkali (e.g., sodium bicarbonate), resulting in a hypochloremic type of alkalosis (p. 341). The Cl is lost by the urinary route, with the excess K mobilized from the intracellular fluid under these circumstances (p. 289).

INCREASED PLASMA SODIUM (HYPERNATREMIA) AND CHLORIDE (HYPERCHLOREMIA)

Theoretically, increase in the concentrations of Na and Cl in the blood plasma may result from: (*a*) loss of water in excess of Na and Cl; (*b*) rigid restriction of water intake without corresponding restriction of NaCl; (*c*) parenteral administration of hypertonic NaCl solution. However, the kidneys are so efficient in preserving the normal composition of the extracellular fluids that hypernatremia, which causes an increase in osmotic pressure of the plasma, is seldom encountered clinically. Its occurrence almost invariably signifies the presence of renal functional impairment in addition to one or more of the factors indicated above.

Sweat is the only body fluid which can be lost in large amounts that is hypotonic, i.e., contains relatively less NaCl than water as compared to the blood plasma. Excessive sweating, therefore, in the absence of corresponding water intake, will tend to produce hyperchloremia and hypernatremia. However, the relative excess of Na and Cl is promptly excreted by the kidneys as long as adequate amounts of water are available for this purpose (i.e., efficient renal excretion). If this condition is allowed to continue, however, the body water available for urine formation is gradually depleted, and oliguria progresses to the point where the renal regulatory mechanisms cannot operate efficiently. Under these circumstances, hypernatremia and hyperchloremia may occur. Usually, excessive sweating, with the attendant intense thirst, leads to the ingestion of large volumes of water. If adequate amounts of NaCl are not taken simultaneously, hyponatremia and hypochloremia may develop (p. 285).

Excessive amounts of water alone may be lost by vaporization ("insensible perspiration") in patients with high fever, in the absence of loss of Na or Cl by other routes (e.g., vomiting, diarrhea). The statements made above, concerning excessive sweating, apply here also. Hyperchloremia and hypernatremia are seldom observed in such cases except in the presence of marked oliguria, and then almost exclusively in infants, in whom the body surface area (for vaporization) : body fluid ratio is relatively high as compared to adults. A similar situation (i.e., water depletion) exists in cases of rigid restriction of water intake, with or without excessive loss.

Increase in plasma Na or Cl or both result from injudicious parenteral administration of hypertonic NaCl solutions in patients with impaired kidney function or with oliguria due to extrarenal deviation of water.

Hyperchloremia (without hypernatremia) may occur in patients in whom the ureters have been transplanted into the bowel (ureterosigmoidostomy), e.g., in bladder carcinoma. If the urine elimination is not maintained by frequent emptying of the bowel, large amounts of NaCl and NH_4Cl may be absorbed, with consequent development of a hyperchloremic type of acidosis (p. 337).

As indicated elsewhere (p. 284), the plasma Cl concentration may be increased in diabetic acidosis due to loss of relatively larger amounts of water than of Cl, particularly in the presence of complicating renal functional impairment, which occurs frequently in this condition.

DECREASED PLASMA POTASSIUM (HYPOKALEMIA; HYPOPOTASSEMIA)

As was indicated in the case of Na, the plasma (serum) K may remain within normal limits in the face of extensive losses of K from the body. It may, in fact, be increased if renal function is impaired in such cases (p. 292). Under these circumstances, which are encountered frequently clinically, the existence of a state of K depletion may be revealed only by the development of hypokalemia after administration of K-free fluids, e.g., NaCl solution, with consequent expansion of the extracellular fluid compartment. As indicated elsewhere (p. 282), loss of excessive amounts of K, per se, results in a hypochloremic type of alkalosis. Therefore, K depletion should be suspected whenever there is an unexplained low plasma Cl and high HCO_3 in the presence of normal kidney function.

Theoretically, hypokalemia may result from: (1) excessive loss of K from the body (e.g., diuresis, vomiting, diarrhea, fistulas, tube drainage of stomach and/or intestine); (2) dilution of extracellular fluids by administration of solutions low in K (usually operating in conjunction with (1)); (3) transfer of excessive amounts of K from extra- to intracellular fluids; (4) inadequate intake of K (usually operating in conjunction with one or more of the aforementioned factors).

Starvation; Malnutrition. A portion of the potassium within the cells is bound to protein, in the proportion of 2.75 mEq. K per gram protein N. In simple protein undernutrition, therefore, one would expect the negative N balance to be accompanied by increased urinary excretion of K in approximately this ratio (mEq. excess K : gm. excess N = 2.75 : 1). However, considerably higher ratios are observed in clinical conditions of malnutrition, even in the absence of such obvious causes of associated dehydration as diabetic acidosis, in which the ratio may be as high as 10 mEq./gm. N.

This excess K loss is due, in part at least, to the fact that the kidneys do not conserve K as efficiently as Na. When subjects on a previously normal intake are suddenly deprived of K, they continue to excrete it in the urine in excess of the calculated K : N ratio. This extra loss may amount to 20 to 50 mEq. or more on the first day, decreasing gradually subsequently, representing loss from intracellular fluids over and above that bound to protein. As indicated elsewhere, this phenomenon is of particular importance in relation to the postoperative management of dehydrated, undernourished patients (p. 290), in whom hypokalemia may be induced by dietary restriction, in addition to injudicious parenteral administration of NaCl and glucose solutions without adequate amounts of K.

Administration of NaCl and Glucose Solutions. When solutions of NaCl and glucose are given parenterally to K-depleted (i.e., dehydrated) subjects, the serum K often falls from the previously normal levels that had been maintained as a result of loss of corresponding amounts of water (p. 283). Expansion of the extracellular fluid volume by isotonic NaCl solution unmasks the state of K-depletion, with consequent hypokalemia, often of serious degree and sudden development.

In addition to this dilution effect, infusion of NaCl solution in such cases results in increased urinary excretion of K. This is due to acceleration of transfer of cell K to the extracellular fluids and its replacement by Na. This process can be reversed only by ad ministration of K (p. 282). The excess mobilized K is excreted in the urine with extra Cl, resulting in a tendency toward a hypochloremic type of alkalosis (p. 282) if renal function is adequate.

Parenteral administration of glucose solution to K-depleted subjects results in increased urinary excretion of K, although not to the same extent as that produced by NaCl. Moreover, the stimulation of cell oxidation processes by the added glucose is accompanied by accelerated passage of K from the extracellular fluids into the cells (p. 32), with consequent fall in the serum K concentration. This is accentuated by simultaneous insulin therapy, and is particularly important in connection with the precipitation of serious hypokalemia during treatment of patients in diabetic coma (p. 290).

Gastrointestinal Disorders. Under normal conditions, of the 70 to 100 mEq. of K ingested daily, 10 mEq. or less are excreted in the feces, the remainder in the urine. Under abnormal conditions, 100 to 200 mEq. or more may be lost via the gastrointestinal tract (e.g., vomiting, diarrhea, etc.). The gravity of this situation is emphasized by the fact that, in such circumstances, renal excretion o K continues, although often at a low level, and that the food intake (and therefore K) is often reduced to practically nothing.

VOMITING. This is perhaps the most frequent cause of K deficiency in surgical cases, together with tube drainage of the gastrointestinal tract, which produces similar effects. Extremely large amounts of water, Na, Cl, and K (p. 280) may be lost by these routes. The extent of depletion of K is increased by the usually simultaneously lowered food intake, the effect of which is discussed elsewhere (p. 288). As in other conditions, the serum K often remains within normal limits until active rehydration with K-free solutions is instituted (pp. 288, 290).

In the event that alkalosis develops as a result of loss of acid gastric juice (p. 341), another mechanism comes into play that tends to further lower the concentration of K in extracellular fluids, including the blood plasma. Alkalosis, per se, may result in passage of increased amounts of K from the cells to extracellular fluids, the excess being eliminated in the urine, with extra Cl. The plasma Cl falls accordingly, and the plasma HCO_3 increases. Thus, in the case of excess vomiting, the state of hypochloremic alkalosis caused originally by loss of gastric HCl is aggravated by added loss of Cl in the urine. Alkalosis of this type cannot be corrected completely by administration of NaCl and NH_4Cl solutions alone; K must be administered if the normal anion : cation (acid base) balance is to be restored.

This effect of alkalosis on the passage of K into the extracellular fluids, with consequent aggravation of the alkalosis, occurs only in the presence of adequate kidney function (p. 282). Inasmuch as renal function is often impaired in alkalosis (p. 167) and dehydration (p. 591), this phenomenon may not be observed until treatment (i.e., NaCl solution) has been instituted, with beginning rehydration and improvement in kidney function.

DIARRHEA. The factors reviewed in discussing the effects of starvation, fluid administration, and vomiting operate also in dehydration due to diarrhea, and to bile, pancreatic, and intestinal fistulas. In these conditions, alteration in acid-base balance is usually in the direction of acidosis (p. 338), i.e., loss of Na in excess of Cl. K-depletion may be severe and may manifest itself in hypokalemia only after rehydration by K-free solutions (p. 288).

An apparently rare condition of congenital alkalosis with diarrhea has been described in infants, in which there is a fundamental defect in intestinal absorption of electrolytes. The voluminous, watery feces contain, in addition to excessive quantities of K, a relative excess of Cl over Na. This tends to produce a state of alkalosis, which induces depletion of intracellular K, as indicated elsewhere

(p. 282). Inasmuch as the excess K is lost in the urine with extra Cl, drawn from the extracellular fluids, the plasma Cl decreases and the HCO_3 increases, aggravating the condition of hypochloremic alkalosis (p. 282).

It is perhaps pertinent to mention here that subjects with alkalosis (e.g., due to vomiting) or hypocalcemia (e.g., in sprue, celiac disease) may not exhibit clinical manifestations of tetany, i.e., neuromuscular hyperirritability, in the presence of hypokalemia. This is perhaps due to the opposing influences of Ca and K ions on this function.

Diabetic Acidosis. In addition to extensive losses of water, Na, and Cl (p. 284), subjects with inadequately controlled diabetes mellitus and increasing acidosis lose large amounts of K in the urine. As acidosis progresses, vomiting occurs commonly, aggravating the K deficiency, which is contributed to also by increased protein catabolism (pp. 77, 288) and reduction in food (and K) intake. Despite this almost invariable state of advanced K depletion, severely dehydrated, acidotic patients in diabetic coma usually present normal or, at times, somewhat elevated serum K concentrations. This is due in part to the associated loss of corresponding amounts of water, and in part to impairment of kidney function, which supervenes frequently (p. 78).

If such patients are treated by parenteral administration of large volumes of NaCl solutions and large doses of insulin, with or without glucose, the serum K concentration usually falls in 8 to 16 hours, often to dangerously low levels. As indicated elsewhere (p. 288), this is due to: (1) expansion of the extracellular fluid volume, unmasking the existing K deficiency; (2) persistence of K excretion in the urine; (3) passage of increased amounts of K into cells from extracellular fluids as a result of stimulation of glucose oxidation and of glycogenesis; (4) retention of increasing amounts of K in the cells as a result of decreased protein catabolism. The development of hypokalemia, with its potentially serious consequences, can be prevented by administration of adequate amounts of K.

Adrenocortical Hormones. Administration of excessive amounts of "salt-active" hormones of the adrenal cortex, notably desoxycorticosterone (p. 432) results in increased urinary excretion of K and the development of a hypochloremic type of alkalosis. This occurs also in Cushing's syndrome, i.e., hypercorticoadrenalism (p. 434). These hormones in some way favor transfer of K from intra- to extracellular fluids, increasing its excretion in the urine, with extra Cl, derived from the extracellular fluids. The plasma Cl concentration falls and the HCO_3 concentration increases correspondingly (i.e., hypochloremic alkalosis).

Postoperative States. Hypokalemia may occur following operations, most often on the gastrointestinal tract, due usually to a combination of circumstances which result in inadequate intake and excessive loss of K. It is favored by a prolonged preoperative period of undernutrition, e.g., due to pain, anorexia, nausea, and by inadequately corrected preoperative dehydration, e.g., due to vomiting, diarrhea, fistula, diuresis. Such individuals are in a state of water, Na, Cl, and K depletion, but, as indicated elsewhere (p. 283), the serum K concentration is usually within normal limits (due to corresponding water loss and, often, to impaired kidney function).

During the first 24 to 48 hours following major operations, there is a rather consistent decrease in urine volume, Na, and Cl, and an increase in urinary K in excess of the amount anticipated on the basis of the negative N balance. These phenomena, which occur also after various types of trauma and other forms of stress, are probably manifestations of an alarm reaction (p. 427), i.e., stimulation of adrenal cortical secretion. This view is supported by the simultaneous occurrence of eosinopenia and increased urinary excretion of adrenocortical hormones (corticoids) during this period. This physiological mechanism would tend to aggravate a pre-existing state of K depletion (i.e., vomiting, diarrhea, starvation, etc.). If, on this background, other factors are superimposed which cause loss of K, hypokalemia may develop. These include: postoperative food restriction (protein and K) (p. 288); tube drainage of the stomach and/or intestine (p. 280); vomiting (p. 289); parenteral administration of large quantities of NaCl and glucose solutions (p. 288).

Persistence and aggravation of K deficiency may lead to troublesome and at times serious consequences. This condition should be suspected under the following circumstances: (1) in the presence of clinical manifestations of hypokalemia (symptoms; ECG changes); (2) when hypochloremic alkalosis appears postoperatively; (3) when a pre-existing hypochloremic alkalosis does not respond satisfactorily to administration of NaCl solution.

Kidney Disorders. The serum K concentration is usually either normal or increased in dehydrated subjects with impaired kidney function occurring either as a result of primary kidney disease or as a consequence of dehydration (p. 591). However, hypokalemia occurs in certain patients with renal disorders, its pathogenesis not being well understood. In some cases it may be due to impaired tubular reabsorption in the presence of adequate glomerular filtration (i.e., "salt-losing nephritis"). However, it has been observed occasionally in uremic patients, i.e., advanced glomerular insufficiency. It has been reported also in patients with congestive heart failure in whom a "low-salt syndrome" developed as a result of active mercurial diuretic therapy (p. 285).

Hypokalemia in renal disease may be contributed to by the use of fluids low in K in artificial kidney devices and for peritoneal lavage or intestinal perfusion, as well as by vomiting and diarrhea, which occur frequently in patients with renal failure.

Although not strictly pertinent here, mention should be made of the induction of K deficiency by prolonged oral administration of K-free cation exchange resins (in treatment of edema).

Familial Periodic Paralysis. In this disorder, attacks of paralysis are associated with sudden drops in serum K concentration, at times to very low levels (e.g., below 2 mEq./liter). Urinary excretion of K decreases simultaneously, which, in the absence of evidence of expansion of the extracellular fluid volume, indicates that large amounts of K must have passed suddenly from the extracellular fluids into the cells. The paralytic attacks may be induced in such cases by administration of glucose, insulin, or epinephrine, which promote the passage of K in this direction.

INCREASED PLASMA POTASSIUM (HYPERKALEMIA; HYPERPOTASSEMIA)

Hyperkalemia is encountered clinically much less frequently than hypokalemia. It occurs when excessive amounts of K enter the extracellular fluids, either from the cells (e.g., in dehydration), or from without (parenteral administration), faster than the excess can be excreted in the urine. In view of the remarkable capacity of normal kidneys for excreting K if sufficient water is available for maintaining an adequate urine volume, hypokalemia seldom occurs spontaneously clinically if renal function and urine volume are satisfactory.

The most common cause is too rapid parenteral administration of solutions containing unduly high concentrations of K to dehydrated subjects with low urine volumes and/or renal functional impairment, due to kidney disease or to the dehydration. This dangerous condition (hyperkalemia) must be carefully guarded against by cautious K therapy under such circumstances, with frequent determinations of serum K concentration and urine output, repeated electrocardiograms, and close clinical observation. If care is not exercised, a condition of K-depletion and hypokalemia may be converted rather quickly to one of hyperkalemia. It should be noted in this connection that bank blood may contain as much as 40 mEq. K per liter of plasma (diffused from the red cells), the concentration increasing with the duration of storage.

The serum K concentration may be elevated in untreated cases of renal failure (uremia), e.g., in chronic glomerulonephritis, pyelonephritis, etc., despite the existence of severe dehydration and K depletion. Hyperkalemia in such cases is due to inadequate renal excretion of the excessive amounts of K passing from cells to extracellular fluid, in part because of progressive dehydration, and in part due to protein malnutrition and excessive protein catabolism. These cell losses are aggravated by fever.

Adrenocortical insufficiency (Addison's disease) results in decreased renal excretion of K (p. 432) (increased excretion of Na, Cl, and water, p. 431). Despite the associated dehydration, however, frank and serious hyperkalemia is seldom encountered in this condition except during periods of crisis (p. 438).

ABNORMAL URINE SODIUM, CHLORIDE, AND POTASSIUM

Most of the important significant changes that occur in urinary Na, Cl, and K in disease are referred to in discussing changes in their concentrations in the blood plasma (pp. 281–292), and require only brief mention here.

Normal women gain weight and retain Na, Cl, and water during the intermenstrual and premenstrual periods. These phenomena, which may be accompanied by latent or frank edema, are probably dependent upon the increased secretion of estrogen and progesterone during these phases of the menstrual cycle. The onset of menstruation is accompanied by increased renal excretion of Na, Cl, and water. Retention of these substances can be induced also by administration of androgens.

In severe, untreated diabetes insipidus the concentrations of Na and Cl in the urine are very low, owing to the large water output. However, except in unusual circumstances, the daily excretion of these elements is not abnormal. In certain instances, administration of large amounts of NaCl is not followed by excretion of a more concentrated urine, as in normal subjects, but rather by the passage of an increased quantity of dilute urine. In some cases, however, the urine NaCl concentration increases if the water intake is restricted simultaneously.

Renal excretion of Na and Cl usually decreases when abnormally large amounts are lost through other channels, e.g., vomiting, diarrhea, tube drainage of the gastrointestinal tract, excessive sweating, etc. This homeostatic mechanism, which tends to preserve the normal electrolyte composition and osmotic pressure of the extracellular fluids, breaks down in the presence of renal functional impairment, due either to kidney disease or to the consequences of dehydration.

In "renal tubular acidosis" (p. 337), excessive amounts of Na, Cl, and K are lost in the urine as a result of inadequate reabsorption due to a defect in the mechanism for acidification of the urine (decreased $Na^+ : H^+$ exchange). Mercurial diuresis, likewise, is accompanied by increased urinary elimination of Na and Cl (decreased tubular reabsorption from glomerular filtrate). Later in the course of these conditions, with marked depletion of body water and electrolytes, there is progressive oliguria and decrease in urine Na and Cl to very small amounts. Urinary excretion of Na, Cl, and K is increased during periods of diuresis and increasing acidosis and dehydration in diabetes mellitus.

Alkalosis, per se, e.g., in prolonged vomiting, or after administration of excessive amounts of sodium bicarbonate, is accompanied by increased urinary excretion of K. This occurs also in adrenocortical hyperfunction (Cushing's syndrome) and after administration of "salt-active" adrenal cortical hormones (p. 432). The use of studies of urinary Cl excretion in the evaluation of adrenocortical function is discussed elsewhere (p. 439). Urinary excretion of K is increased in dehydrated (K-depleted) subjects by administration of K-free solutions of NaCl and of glucose (p. 288).

Excretion of Cl in the urine diminishes sharply during the course of pneumococcus pneumonia and other infectious diseases accompanied by hypochloremia (p. 285). Urine Na and Cl decrease (also urine volume) during the first 24 to 48 hours after major operations; the urine K increases simultaneously (p. 290).

Renal excretion of K falls off sharply during paralytic episodes in familial periodic paralysis, simultaneously with the drop in serum K concentration.

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CHAPTER XIV

Water Balance

A comprehensive consideration of the subject of water balance is beyond the scope of the present discussion. However, an understanding of certain of the fundamental factors involved in the maintenance of normal water balance is essential for the proper understanding of certain chemical changes which may occur in the body fluids under abnormal conditions.

In the normal adult organism, not undergoing changes in weight, the quantity of water supplied daily is balanced by that eliminated, a state of equilibrium being maintained.

WATER INTAKE

Water is supplied to the body from the following sources: (*a*) dietary liquids; (*b*) solid food; (*c*) oxidation of organic foodstuffs.

Water comprises 70 to 90 per cent of the weight of the average diet of adults, even apparently very solid foods consisting largely of water. Moreover, water is one of the chief products of combustion of protein, fat and carbohydrate in the body, the quantities produced by oxidation of 1 gm. of each of these substances being as follows: protein, 0.34 cc.; fat, 1.07 cc.; carbohydrate, 0.56 cc. It may be calculated that 10 to 15 cc. of water are formed per 100 calories of energy produced. An ordinary 3000 calorie diet therefore contains about 450 cc. of water in the solid food and may provide an additional 300 to 450 cc. of water of oxidation. The remainder of the water intake is supplied by dietary liquids (Fig. 27).

WATER OUTPUT

Water leaves the body in the (*a*) urine, (*b*) feces, (*c*) perspiration, and (*d*) so-called insensible perspiration (evaporation from skin and lungs) (Fig. 27).

Feces. Under normal conditions, the 3000 to 8300 cc. of digestive fluids (Table 14) entering the alimentary tract are almost completely reabsorbed in the intestine. On an ordinary mixed diet, about 80 to 150 cc. of water are excreted daily in the feces of normal adults. This may be increased considerably on a high vegetable diet and particularly in the presence of diarrhea.

Insensible Perspiration. In the absence of active perspiration, the body is continually losing water vapor from the skin surface and lungs in inverse proportion to the relative humidity of the atmosphere. Inasmuch as 0.58 calories are absorbed in the vaporization of 1 cc. of water, the heat lost by this process, termed "insensible perspiration," at ordinary room temperatures amounts to about 25 per cent of the total heat loss. The latter is proportional to the quantity of heat produced, which is a function of metabolism. The quantity of water lost by insensible perspiration is therefore an obligatory loss, determined by metabolic activity and, in a normal adult, may be calculated as 500 cc. per sq. meter of body surface area per day. This amounts to about 850 cc. for a 70 kg. man (1.73 sq. meters).

Table 14. Normal Quantitative Water Turnover in 70 Kg. Subject
(After Adolph, E. F.: Physiol. Rev. *13*:336, 1933.)

FLUIDS EXCRETED AND REABSORBED	VOLUME CC.
Saliva	500– 1,500
Gastric secretion	1,000– 2,400
Intestinal secretion	700– 3,000
Pancreatic secretion	700– 1,000
Liver bile	100– 400
	3,000– 8,300
FLUIDS LOST FROM THE BODY	
Urine	600– 2,000
Feces	50– 200
Insensible perspiration	350– 700
Perspiration	50– 4,000
Milk	0– 900
	1,050– 7,800
Total fluid turnover	4,050–16,100

Perspiration. When either heat production or the environmental temperature rises to the point where heat loss by the ordinary means is inadequate, the sweat glands become active. Obviously, the quantity of water lost by this route varies enormously. Moreover, in contrast to insensible perspiration, which consists solely of water, sweat contains 30 to 90 mEq./l of Na and of Cl (Fig. 31, p. 313). It is a hypotonic solution, plasma containing about 140 mEq./l of Na. Sweating therefore removes from the body relatively more water than electrolytes.

Urine. The urine is the important medium of elimination of water provided to the body in excess of its fixed requirements. The kidneys have a remarkable capacity for regulating urinary excretion of water regardless, within wide limits, of the simultaneous requirement for excretion of solids (p. 570). However, their ability to concentrate is not unlimited, a certain minimal quantity of water being required for excretion of a given amount of solute. On an average adequate diet (adults), providing about 50 gm. of solids for daily urinary excretion, a minimum of approximately 500 cc. of water (300 cc./sq. meter body surface) is required for their solution. Failure to meet this obligatory urine volume requirement will result in retention of certain urinary constituents, mainly urea, in the body fluids.

The quantitative turnover of water by various organs and the amount excreted in normal circumstances are presented in Table 14 and Figure 27. When one considers that all of the secretions and excretions mentioned are derived directly from the blood plasma, it appears that a total daily fluid turnover of 4000 to 16,000 cc. is accomplished from a circulating medium of about 3500 cc. (plasma volume). This phenomenon is rendered possible by (1) the efficient and rapid reabsorption of the large volumes of fluid secreted into the gastrointestinal tract (3000–8300 cc.); (2) the presence of a large and readily available reservoir represented by the interstitial fluid compartment (about 10,500 cc.); (3) the vast area of the capillary bed of the body (about 68,000 sq. ft.), which constitutes an enormous filtration surface for the rapid interchange of fluid between the vascular and interstitial fluid compartments.

Equilibrium Requirements. As indicated above, in addition to the small amount excreted in the feces (80–150 ml.), the minimal daily water requirement is fixed by certain metabolic requirements: (*a*) loss of heat by insensible perspiration (normally about 850 cc.); (*b*) renal excretion of excess solid material, mainly urea, and therefore determined largely by the protein intake (normally about 500 cc. of water). Consequently on an average normal diet, approximately 1500 cc. of water must be available for elimination by these routes if the body temperature and blood urea N are to be maintained within normal limits. The "solid" portion of such a diet contributes about 800 cc. of water, approximately one-half of which is pre-formed, the remainder being produced in the course of oxidation of the organic foodstuffs in the body. The balance of the water requirement must be supplied by intake of liquids if the body fluids are to be maintained at a normal level.

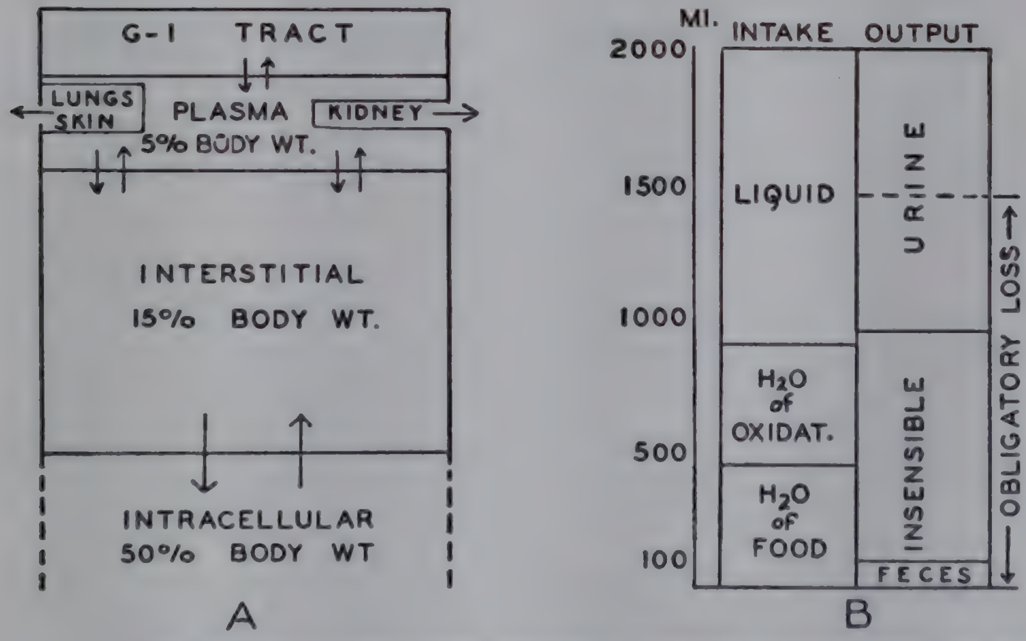

Fig. 27. *A*, body water compartments. *B*, sources of water to the organism, and water excretion in a 70-kg. man on an average diet (after Gamble).

BODY FLUID COMPARTMENTS

Water comprises approximately 70 per cent of the adult body weight and may be regarded as existing in two main compartments, (1) extracellular (20% body weight) and (2) intracellular (50%). Extracellular water is further subdivided into (*a*) blood plasma water (5% body weight) and (*b*) interstitial fluid (15%).

These fluid compartments are illustrated in Figure 27. Water entering the organism, i.e., from the gastrointestinal tract, passes into the blood stream, an equivalent amount leaving the latter by way of the urine, lungs, skin and feces. The water of the plasma is in equilibrium with that of the interstitial fluid, and the latter with the intracellular water, across the boundaries between these compartments, i.e., the capillary walls and the cell membranes, respectively. The interstitial fluid, serving as a sort of middle-man for the other two fluids, acts also as a buffer which prevents rather sudden changes in composition of the plasma, due to absorption from the intestine, from being reflected directly in the intracellular fluid. Moreover, being a rather elastic compartment, it can expand or contract considerably, in conditions of excessive retention (edema) or loss (dehydration) of fluid, in the absence of comparable or even significant alterations in the volume of plasma or intracellular fluid, which must be maintained rather rigidly in the interests of normal circulation and cell function.

Volume of Body Fluid Compartments

The volume of (*a*) the circulating blood, (*b*) the plasma, (*c*) total extracellular fluid, and (*d*) total body water could be measured by introduction of a substance into the body if that substance would fulfill the following requirements: (1) be retained exclusively in the fluid compartment in question; (2) not leave that compartment during the test period; (3) distribute itself uniformly throughout that compartment; (4) be capable of precise quantitative determination in the blood or plasma. A number of methods have been proposed, none of which is entirely satisfactory; certain of them, however, have proved useful.

Blood and Plasma Volume. (*a*) CARBON MONOXIDE METHOD. Carbon monoxide displaces oxygen from hemoglobin, volume for volume, and can be measured accurately in blood, either as CO or as HbCO. The subject breathes a known amount of CO and the concentration of HbCO in the blood is determined after allowing sufficient time for mixing. If the total hemoglobin concentration is known, one can calculate how much CO would be required to saturate the Hb to its full capacity and can arrive at the total blood volume. By determining the hematocrit value (relative volumes of packed cells and of plasma), the plasma volume can be derived.

(*b*) RADIOACTIVE IRON METHOD. Fe^{59} is administered to a normal or anemic subject; it becomes incorporated in the Hb of the circulating erythrocytes. A predetermined amount of the labeled red cells is injected intravenously in the test subject (compatible blood). Measurement of radioactivity in blood samples withdrawn after allowing time for mixing indicates the extent of dilution of the injected cells and permits calculation of the volumes of blood and plasma (from hematocrit value).

(*c*) DYE METHODS. The dye employed most commonly is Evans blue

(T-1824). A known amount is injected intravenously and, after allowing time for distribution throughout the blood plasma, a sample of oxalated blood is withdrawn, centrifuged, and the concentration of dye in the plasma determined colorimetrically. The extent of dilution of the quantity injected can be calculated (plasma volume) and, knowing the hematocrit value, the total blood volume can be determined.

The CO method requires cooperation of the subject, which cannot always be secured under clinical conditions. Moreover, other technical difficulties introduce the possibility of serious errors. Furthermore, the accuracy of this procedure, as well as the Fe^{59} method, depends upon uniformity of distribution of all of the "labeled" erythrocytes in the circulation. This cannot always be assured, especially in disease states (e.g., shock), in which variable and undeterminable numbers of cells may be "segregated" in such organs as the spleen or in the splanchnic bed. Inaccuracies in the dye method arise chiefly out of the fact that a variable and undeterminable portion of the dye may leave the blood stream, particularly into the hepatic lymphatic system, which may itself vary in disease states. Correction must be made for excretion in the urine. It gives values for plasma volume about 15 per cent higher than those obtained with the CO method. In addition, calculations of total blood volume on the basis of factors measured in either cells or plasma is subject to inaccuracies arising out of variations in the ratio of cells to plasma in different portions of the circulation.

Generally acceptable average values for blood and plasma volume in normal subjects are as follows: (1) whole blood, 2500 to 3200 cc./sq. meter body surface, or 63 to 80 cc./kg; (2) plasma, 1300 to 1800 cc./sq. meter, or 35 to 45 cc./kg. Values for whole blood are about 7 per cent higher for men than for women, but the plasma values are approximately the same.

A slight increase in blood and plasma volume occurs in normal pregnancy, the increase in plasma volume being relatively greater than that in total blood volume. This disproportionate increase in plasma volume is responsible in part for the physiological decrease in red blood cell count and plasma albumin concentration during this period (p. 158).

Total Extracellular Fluid Volume. A number of substances, when injected intravenously, cross the capillary walls but not the cell walls generally, being distributed uniformly throughout both plasma and interstitial fluids (extracellular fluid compartment). These include sucrose, sulfocyanate, mannitol, sulfate, bromide, and sodium (radioactive). They should lend themselves, therefore, to calculation of the extracellular fluid volume by much the same procedure as that employed in the dye method for plasma volume, allowing more time for complete mixing and correcting for excretion in the urine. Sucrose, mannitol, sulfate and radioactive sodium appear to be the most satisfactory for practical purposes, the others entering erythrocytes and digestive secretions (sulfocyanate) to a certain extent. Employing these methods, values have been obtained for total extracellular fluid volume approximating about 20 per cent of the body weight. Of this, about one-fourth is blood plasma and three-fourths is interstitial fluid (5 and 15%, respectively, of body weight).

Total Body Water. There is no accurate method for determining total body water, inasmuch as no measurable substance has yet been found which, on injection, is distributed uniformly throughout the entire body water and undergoes no metabolic change during the test period. Heavy water, antipyrine, thiourea, and sulfonamides have been suggested. Although not entirely free from error, heavy water and antipyrine may be satisfactory for clinical purposes.

The water content of several tissues has been determined by direct measurement (animals and human biopsy or autopsy material). Water comprises approximately 70 per cent of the body weight of adults (75% in children), varying considerably in different tissues. Intracellular water comprises about 50 per cent of the body weight.

Composition of Body Fluid Compartments

Inasmuch as water serves chiefly as a relatively inert medium in which are conducted the chemical reactions constituting living processes, its significance must be considered in relation to the other components of the body fluids which, in fact, largely determine its volume and distribution in the organism. The marked differences in composition of the intracellular and interstitial fluids and the less striking differences between the latter and the blood plasma are due to differences in permeability of the membranes separating these compartments to the solutes which these fluids contain.

Milliequivalents (mEq.). Elements which combine chemically with one another do so in fixed weight ratios, which are functions of their atomic weights and valences. Thus, 1.008 gm. of hydrogen (at. wt. 1.008) combines with 8 gm. of oxygen (at. wt. 16) to form water, and with 35.457 gm. of Cl (at. wt. 35.457) to form HCl. Similarly, 35.457 gm. of Cl combines with 22.997 gm. of Na, 39.096 gm. of K and 20.04 gm. of Ca (at. wt. 40.08). These numerical values are designated "equivalent weights," because they are equivalent in their combining power. An "equivalent weight" may be defined as the weight of a molecule, or of an atom or radical (group of atoms reacting chemically as a unit), divided by its valence. A "milliequivalent" is 1/1000 of the gram equivalent weight, or the latter expressed in milligrams.

The concentrations of the solid constituents of the body fluids may be expressed in terms of weight per unit volume (e.g., milligrams per 100 cc.) or in terms of chemical combining power (e.g., milliequivalents per liter) (mEq./l.). The former may be converted to the latter according to the following formula:

$$\text{mEq./l.} = \frac{\text{mg./l. } (= \text{mg./100 ml.} \times 10) \times \text{valence}}{\text{atomic or radicular weight}}.$$

Osmolar Concentration. All body fluids, extracellular and intracellular, possess one physicochemical property in common, i.e., an equal osmotic pressure. Osmotic pressure is a function of the concentration of active chemical components in a solution, i.e., the number of ions and mols (undissociated compounds). The designation "osmol" is applied to one chemically active mol or ion.

Consequently, solutions of the same osmotic pressure have an equal total osmolar concentration of solutes.

A solution containing 1 osmol (expressed in grams) per liter of water depresses the freezing point of the water by 1.86° C. The freezing point of blood plasma is about −0.56°. It must, therefore, contain osmotically active solutes in a total concentration of 0.56/1.86 or 0.301 osmols per liter plasma (320 milliosmols/liter of water), of which non-electrolytes contribute about 10 milliosmols.

In this connection, the components of the body fluids may be classified conveniently as follows:

(1) Compounds of very large molecular size, e.g., proteins, lipids, glycogen, to which all but a few capillary and cell membranes are almost completely impervious. Inasmuch as osmotic pressure is proportional to the number of active chemical components per unit water, these compounds, because of their large molecular size, exert relatively little effect on osmotic pressure in proportion to their concentration on a weight basis. For example, the concentration of plasma proteins, about 7 gm. per 100 cc. of plasma, with molecular weights of about 70,000 to over 1,000,000, represents only about 2 milliosmols per liter. Moreover, since these large compounds displace a large volume of water and the osmolar concentration is expressed in terms of units per liter of water, correction must be made for this displacement. In the case of plasma, this correction factor is 0.93, the total osmolar concentration being 301 milliosmols per liter of plasma (320 milliosmols per liter of plasma water).

Table 15. Radicular and Equivalent Weights of Important Body Fluid Electrolytes

ELEMENT OR ION	VALENCE	ATOMIC OR RADICULAR WEIGHT	EQUIVALENT WEIGHT
H	1	1.008	1.008
Na	1	22.997	22.997
K	1	39.096	39.096
Cl	1	35.457	35.457
Ca	2	40.08	20.04
Mg	2	24.32	12.16
HCO_3	1	61.018	61.018
HPO_4	2	95.988	47.994
H_2PO_4	1	96.996	96.996
SO_4	2	96.066	48.033

(2) Electrolytes, which cross capillary walls freely, but to certain of which various cells membranes exhibit a variable degree of impermeability under different conditions. Electrolytes dissociate into ions and therefore exert an osmotic pressure greater than their molar concentrations because the ions behave as units. The osmolar concentration of NaCl is practically twice its molar concentration because it dissociates almost completely into Na^+ and Cl^-. One millimol of $CaCl_2$ represents three milliosmols, dissociating into $Ca^{++} + 2Cl^-$. In the presence of large amounts of protein, certain elements, e.g., Ca and K, may exist as relatively poorly dissociated salts of protein, being osmotically active only to the extent of the degree of dissociation.

(3) Organic compounds of a molecular size which permits their free diffusion across capillary walls, e.g., urea, glucose, amino acids. Some cell membranes may be relatively impermeable to certain of these, as to certain electrolytes.

EXTRACELLULAR FLUID

All body cells exist in an environment of fluids collectively designated extracellular fluid. These include the blood plasma, interstitial fluid, lymph, and peritoneal, pericardial, pleural, joint and cerebrospinal fluids. These fluids have an essentially uniform qualitative composition and what quantitative differences exist are due chiefly to differences in the concentrations of protein, which range from about 7 per cent in plasma and but slightly less in hepatic lymph, to less than 0.1 per cent in cerebrospinal fluid. They are solutions chiefly of NaCl and $NaHCO_3$, with small amounts of Ca, Mg, K, H, phosphate, sulfate and organic acid ions, variable amounts of protein, some nonelectrolytes (glucose, urea, lipids, etc.), and with pH values ranging from 7.35 to 7.45 under normal conditions.

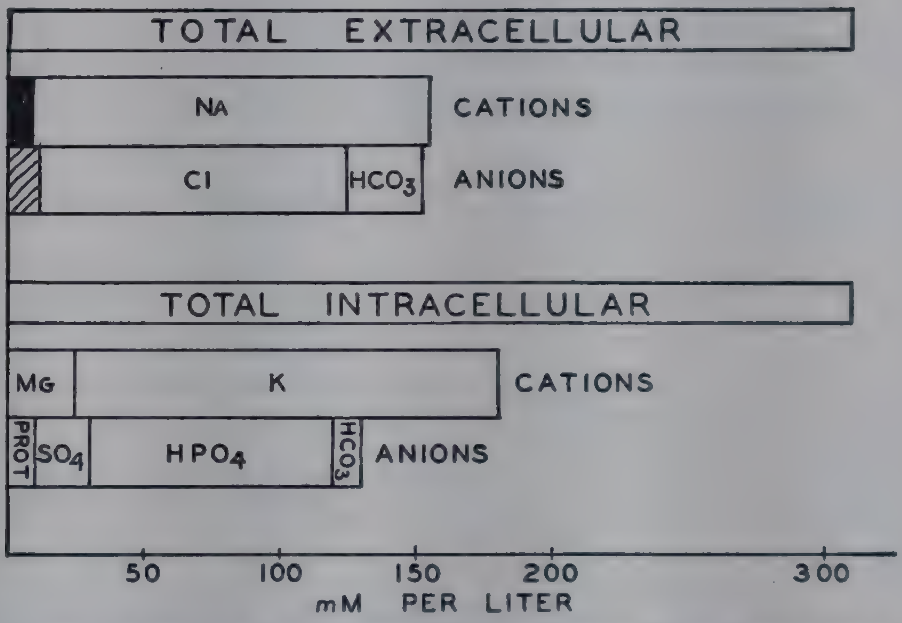

Fig. 28. Osmolar equality of the extracellular and intracellular fluids (after Gamble).

The total concentration of the ionic constituents is about 310 mEq. per liter of plasma (about 335 mEq. per liter of plasma water). In accordance with the laws of electrical neutrality, cations and anions each comprise half of the total concentration, expressed in terms of chemical equivalence. Proteins, being amphoteric, act as anions in these slightly alkaline fluids, the chemical structures of which are indicated in Figure 29.

The milliequivalent value for protein is obtained by multiplying grams of protein per 100 ml. by 2.43 (Van Slyke factor). Each millimol (mM) of protein represents about 8 milliequivalents (mEq.), which accounts chiefly for the discrepancy between the ionic osmolar and equivalent values for plasma. At the pH of extracellular fluid, 80 per cent of the phosphate radical carries two equivalents (B_2HPO_4) and 20 per cent one equivalent of cation (BH_2PO_4). The valence of phosphate is therefore calculated as 1.8. The commonly used designations of carbonic acid and bicarbonate values in terms of "volumes per cent CO_2" may be converted to milliequivalents per liter by dividing by 2.22.

The relative importance of the several components of the extracellular fluids in preserving osmotic and anion-cation (acid-base) balance is indicated by their osmolar and equivalent concentrations, respectively (Fig. 29; Table 11). However, those components, particularly cations, which are present in comparatively very low concentrations, viz., K^+, Ca^{++}, Mg^{++}, and H^+, exert profound influences upon a variety of physiological processes. Therefore, relatively slight deviations from their normal concentrations produce significance biological effects.

As illustrated in Figure 29, the chief structural difference between the two compartments of extracellular fluid, i.e., blood plasma and interstitial fluid, is the relatively large amount of protein in the former. The presence of this non-diffusible component results in certain readjustments of the diffusible ions in order to maintain anion-cation equivalence (Donnan equilibrium, p. 305). Consequently, the interstitial fluid contains a somewhat higher total concentration of diffusible anion and a lower concentration of cation than does the plasma.

INTRACELLULAR FLUID

In contrast to the rather complete and precise information available regarding the composition of extracellular fluids, data regarding the chemical structure of intracellular fluids are fragmentary and incomplete. It seems obvious that differences in structure and function of cells of various tissues might be reflected in differences in their chemical constitution, in contradistinction to the uniformity of composition of extracellular fluids. Sufficient information is available, however, particularly on erythrocytes and muscle cells, to indicate a rather general and more or less consistent pattern of differences between the two major fluid compartments (Figs. 28, 29).

Whereas Na is the major cation in the extracellular fluid (142 mEq./liter), much smaller amounts are present in the intracellular fluids (0–40 mEq./liter), which contain also little or no Ca. The chief cations of the latter fluids are K, about 140 mEq./liter in muscle, and Mg, about 40 mEq./liter in muscle (5 and 3 mEq./liter respectively in plasma). As regards anions, the intracellular fluids contain much more phosphate and sulfate ions and protein than the extracellular fluids. Cl, the major anion of the latter, is practically absent from the former, except in the case of erythrocytes (Fig. 29), and cells of the kidney tubules, stomach and intestines. Cells of the latter organs contain Na and Cl, since they are engaged in reabsorption or secretion of these elements. However, in these situations, Na and Cl are apparently not in diffusion equilibrium with the extracellular fluid. HCO_3^- is the only ion which exists in both fluids in concentrations of even approximately comparable magnitude. The marked differences in concentration of Na and K in the extra- and intracellular fluids indicate a certain degree of impermeability of the cell membranes to these ions. This impermeability is, however, not absolute nor fixed, both ions being able to cross the membrane more freely under certain physiological and pathological conditions (p. 278). It

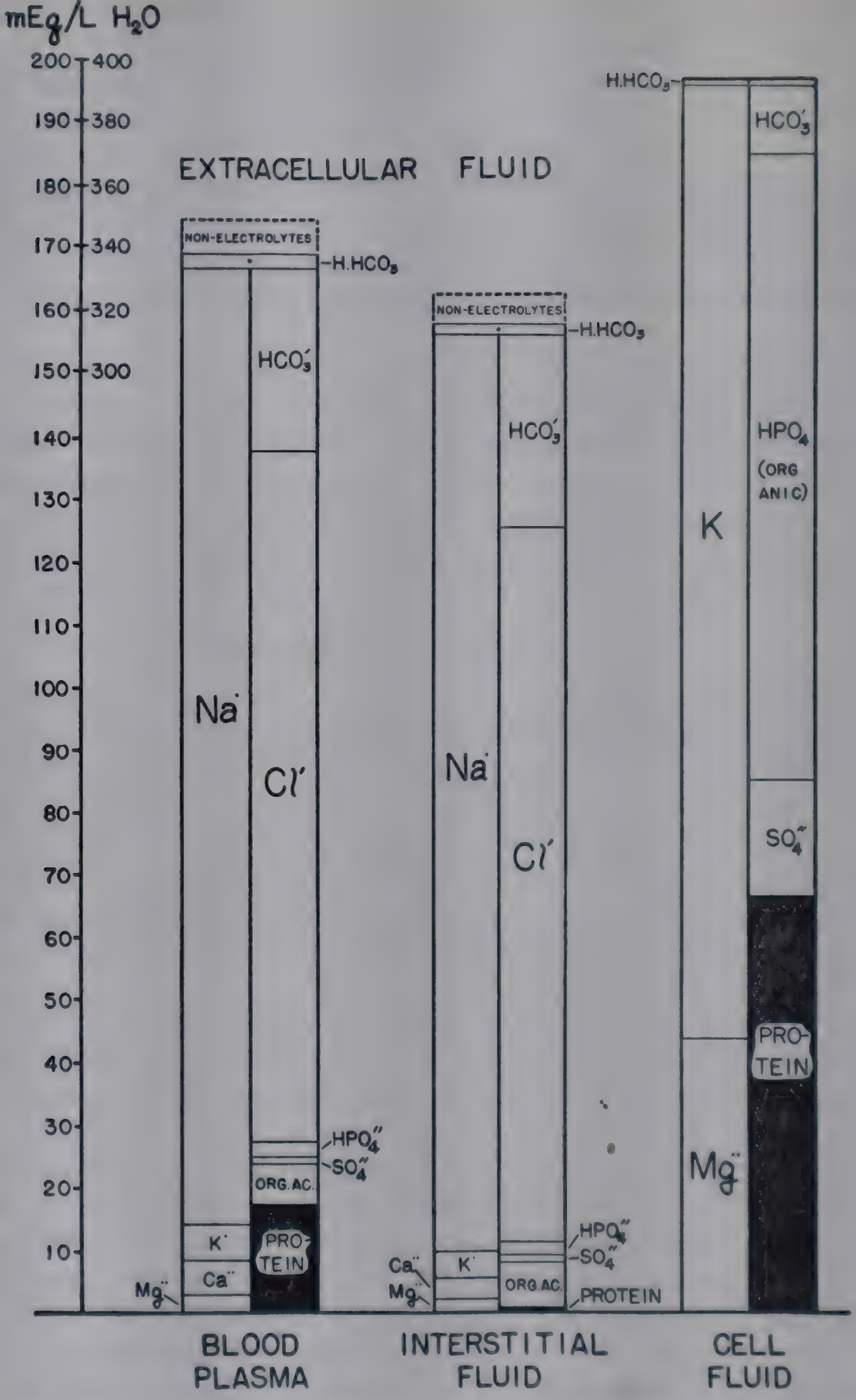

Fig. 29. Composition of intracellular and extracellular fluids. (From Gamble: Chemical Anatomy, Physiology, and Pathology of Extracellular Fluid, Harvard University Press, 1950.)

would appear that as K leaves the cells in increased amounts, Na enters, the total cationic concentration being thereby maintained approximately, but not perfectly (p. 282).

The bulk of the phosphate is in organic combination and the extent of dissociation of these compounds is not known. Similarly, much of the Mg and perhaps of the K is undoubtedly present as undissociated salts of protein and organic phosphate and, therefore, is not in ionic form. Furthermore, the concentration of protein ions has not been established accurately and the estimated HCO_3^- concentration is subject to correction for carbamino CO_2 (p. 359). These factual deficiencies contribute to the present uncertainty regarding the constitution of the intracellular fluids on an osmolar and equivalent basis.

EXCHANGES BETWEEN FLUID COMPARTMENTS

The continual entrance into the body of substances from without (oxygen, water, organic and inorganic foodstuffs, etc.) and production by the cells of a great variety of metabolites, many of which must be distributed to other tissues or be excreted, implies a continual movement of various components of the body fluid compartments across the boundary membranes (capillaries and cell walls). The most important of these exchange systems may be outlined as follows:

(1) *Alveolar Air: Blood Plasma* (p. 353). This system provides for entrance of oxygen into and loss of CO_2 and water from the body.

(2) *Plasma: Erythrocyte* (p. 326). This system provides for ready exchange of oxygen, CO_2, water and certain anions (particularly Cl^- and HCO_3^-) in both directions. Cations are exchanged very slowly.

(3) *Plasma: Interstitial Fluid.* These two media are separated by the capillary walls, which are perfectly permeable to water, inorganic ions and small organic molecules (e.g., glucose, amino acids, urea, etc.) but not to large organic molecules, such as proteins.

(4) *Interstitial Fluid: Intracellular Fluid.* These two compartments are separated by the cell membranes, across which gases, water, and small, unchanged molecules can diffuse readily, but, at least under normal conditions, cations or anions cannot, at least not freely. These membranes are also impermeable to large molecules, such as proteins, except in special situations, viz., the liver.

The first two of these systems are discussed in the section on respiration (pp. 356, 359), since they are intimately concerned with the transport and exchanges of oxygen and CO_2.

Gibbs-Donnan Equilibrium. If two solutions are separated by a membrane which is freely permeable to the solvent and solutes, the concentrations of the latter will be identical when equilibrium is established. Thus, both the chemical composition and the osmotic pressures of the two solutions will be the same.

If, on the other hand, one of the solutions (solution I) contains an electrolyte, e.g., NaCl, to which the membrane is permeable, while the other (solution II) contains a substance, e.g., Na proteinate, in which the anion (protein) is too large to pass through the membrane, the distribution of Na^+ and Cl^- at equilibrium will be unequal on opposite sides of the membrane. Since electrical neutrality must be maintained, in solution II the cation Na^+ must balance two anions, Cl^- and protein$^-$, whereas in solution I it balances only Cl^-. It follows that the concentration of Na will exceed that of Cl in solution II, whereas they will be equal in solution I.

Certain quantitative ionic relations for the Gibbs-Donnan equilibrium may be stated as follows:

1. The concentration of a diffusible cation (e.g., Na) is higher and that of a diffusible anion (e.g., Cl) lower on the side of the membrane (e.g., capillary wall) containing a nondiffusible anion (e.g., protein).

2. At equilibrium, if the volume of the solvent is kept constant, the sum of the concentrations of diffusible ions in the solution containing the nondiffusible ion exceeds the sum of the concentrations of the same ions in the other solution.

3. The total ionic concentration in the former solution (i.e., with nondiffusible ion) exceeds that in the latter. Consequently, the former will have a higher osmotic pressure than the latter, determined not only by the concentration of the nondiffusible ion (colloid osmotic pressure), but also by the higher concentration of diffusible ions.

The Gibbs-Donnan effect is of great physiological significance in biological systems involving ion exchanges across permeable membranes, especially when the fluid on one side of the membrane contains a nondiffusible component, e.g., protein, in high concentration. It finds particular application in exchanges of Cl^- and HCO_3^- between the blood plasma and erythrocyte and in water and ion exchanges between the blood plasma and interstitial fluid (i.e., across the capillary wall).

Plasma : Interstitial Fluid Exchange. Exchanges between the blood plasma and interstitial fluid occur across the endothelial lining of the capillaries, which act as semipermeable membranes, allowing free passage of water and crystalloid solutes, inorganic and organic, but not of colloids of large molecular size, viz., proteins. This impermeability to proteins is not absolute nor uniform, the concentration of protein in interstitial fluids varying from 0.05 to 0.5 per cent in subcutaneous tissues and serous cavities to 4 to 6 per cent in liver. The concentrations of diffusible electrolytes on the two sides of the capillary will vary, their distribution depending upon the difference in protein concentration in the two fluids (Gibbs-Donnan effect). The osmotic pressure will be greater in the plasma than in the interstitial fluid, due both to the higher protein concentration (colloid osmotic pressure; C.O.P.) and to the consequent higher concentration of diffusible ions. Such freely diffusible organic solutes as urea, glucose, creatinine, etc., being nonionized, are not subject to the Donnan effect and are distributed equally through the body water. Their diffusion across semipermeable membranes is therefore determined solely by their concentration gradients.

In the absence of opposing forces, water and crystalloid solutes will tend to pass from the interstitial fluid to the plasma, which contains protein in higher concentration, thereby producing an osmotic pressure gradient. This tendency may be counterbalanced by the opposing force of the capillary blood pressure and may also be modified by the concentration gradients of the individual solutes.

The colloid osmotic pressure (C.O.P.) of the proteins of normal plasma is about 22 mm. Hg (30 cm. H_2O). The capillary blood pressure varies considerably in different tissues, but is higher than the C.O.P. at the arterial end of the capillary and lower than the C.O.P. at the venous end. Consequently, filtration from the plasma occurs at the arterial end and reabsorption into it at the venous end of the capillary. Moreover, the increasing concentration of nondiffusible colloids (viz., proteins) which results from loss of plasma water creates a relatively small but actual gradient of C.O.P., which further favors reabsorption as the blood pressure falls in the distal end of the capillary. This mechanism (Starling hypothesis) provides for a continual circulation of fluid between the capillaries and the tissue spaces, a balance being maintained between the quantity of water filtered and that reabsorbed. Exchanges of diffusible solutes between these two fluids depends upon these circumstances and also, independently of them, upon their individual concentration gradients.

Filtration from the capillaries is opposed by the tissue tension, which varies considerably in different situations, being relatively low in loose areolar tissues (e.g., eyelids, external genitalia) and high in dense tissues (e.g., muscle, liver, etc.). Fluid leaves the tissue spaces not only by direct reabsorption in the blood plasma but also by filtration across the lymph capillaries, ultimately reaching the venous blood by way of the lymphatic circulation.

Interstitial Fluid : Intracellular Fluid Exchange. Exchanges between these two compartments occur across cell membranes, which are generally freely permeable to gases, water and small uncharged molecules (urea, etc.) but not to large colloidal molecules, such as proteins. The enormously higher concentration of proteins and other colloids in the intracellular than in the interstitial fluids would cause a much greater osmotic pressure within the cells (Gibbs-Donnan effect), were it not for the fact that the cell membranes are generally not freely permeable to inorganic ions, especially cations. Consequently, the electrolyte composition of cells is quite as distinctive as their organic constitution (Fig. 29, p. 304). Because of this relative impermeability to inorganic ions, adjustment to deviations of osmotic pressure is usually accomplished largely by transfers of water, not by exchanges of inorganic constituents, the freely diffusible organic solutes (urea, creatinine, etc.) moving with the water. Inasmuch as the concentrations of protein in the body fluids are more stable than the concentrations of inorganic ions, the latter constitute the dominant factor in determining the total osmotic pressure and the exchanges of water between the body fluid compartments. The milliequivalent value of the extracellular fluids (310 mEq/l.) approximates their milliosmolar value (Fig. 28) because of the enormous preponderance of univalent ions. This is not true, however, of the intracellular fluids, the total milliosmolar value of which is the same as that of extracellular fluids, but the milliequivalent value of which is considerably greater (about 390), because of the relatively high concentrations of multivalent ions (Mg^{++}, $SO_4^{=}$, $HPO_4^{=}$, protein).

When Na is introduced into the blood plasma, it is distributed rapidly throughout the extracellular fluids, but penetrates the cells only very slowly. Consequently, an increase in Na concentration in the extracellular fluids will cause water to pass out of the cells, equalizing the osmotic pressure in the two fluid compartments. Withdrawal of Na from the extracellular fluids causes passage of water in the opposite direction, i.e., into the cells.

Shifts in water are also caused by changes in pH of the body fluids. The explanation frequently given is that acids remove cations from the weakly dissociated proteins, forming more completely dissociated salts and thereby increasing the number of osmotically active components. Inasmuch as the protein content of intracellular fluids is greater than that of extracellular fluids, acidification, according to this view, results in greater increase in osmotic pressure within the cells than in the extracellular fluids. Consequently, water passes into the cells under such circumstances. This explanation is not entirely satisfactory from a chemical standpoint.

The relative impermeability of cell membranes to inorganic ions is not uniform or constant. HCO_3^- ions are generally freely diffusible; Cl^- ions pass freely into and out of erythrocytes (p. 327) and the acid-secreting cells of the gastric mucosa. Although permeability to Na^+ and K^+ ions is restricted, Na^+ may enter cells in increased amounts when they lose K^+ and the passage of K^+ across cell membranes appears to be related to metabolic activities of the cells. For example, there is acceleration of the entrance of K^+ into muscle cells during muscular activity and into other cells during periods of administration of glucose or insulin (p. 32). Changes in the distribution of Na^+ and K^+ can be produced by adrenal cortical hormones (p. 431) and may occur also in dehydration (p. 279ff.) and acidosis (p. 282).

Phosphate is present within cells in much larger amounts than in extracellular fluids. Much of this is in organic combination, e.g., as hexose and triose phosphates, nucleotides, nucleic acids, phosphocreatine (muscles), etc. However, the concentration of inorganic phosphate, too, is higher than in the extracellular fluids and fluctuates continually in relation to the latter at different levels of cell metabolic activity involving formation and splitting of phosphorylated metabolites. In contrast to the case of K^+, the rate of entrance of phosphate into muscle cells is not increased significantly by muscular activity but is increased markedly during the recovery period. It is increased also by administration of glucose and insulin (p. 32).

Considerations such as these have led to the conclusion that many of the inorganic cations and anions do not enter and leave the cells by simple diffusion, but that the cell membrane participates actively in such transfers. Active transport of ions across these membranes implies the performance of work to overcome differences in concentration or electrochemical potential. It is probable that the energy requirements for these purposes are derived from cellular metabolic reactions to which the transferred ions are related or in which they are involved. The concentrations of K, Na, Mg, Ca and phosphate in the cell can vary, within limits, in accordance with the metabolic activity of the cell, without influencing exchanges of water, because, presumably, considerable fractions of these substances form undissociated or poorly dissociated combinations with cell proteins and are therefore osmotically inactive.

ABNORMAL BLOOD VOLUME

Pathologic variations in total blood and plasma volumes may be dependent upon primary changes in the number and volume of the corpuscles. Thus, in polycythemia vera the enormous increase in red blood cells may result in a marked increase in total blood volume with a normal, increased or decreased plasma volume. On the other hand, a marked diminution in the number of red blood cells may be accompanied by a decrease or normal total blood volume and a normal, increased or decreased plasma volume. Moreover, in the presence of increased acidity of the blood, the corpuscles tend to swell, due to the imbibition of water, at the expense of the plasma volume; decreased acidity of the blood tends to produce the opposite effect.

Increased Blood Volume. An actual increase in total blood volume in relation to body weight or surface area is seldom encountered clinically. It occurs in polycythemia vera, at times in chronic leukemias before the development of anemia, occasionally during periods of water intoxication in diabetes insipidus and, similarly, in experimental water intoxication produced by excessive water administration plus the administration of pituitrin. In the last condition, the increase is due to a primary increase in plasma volume. A temporary increase in total blood volume, due to an increase in plasma volume, may occur immediately following the administration of hypertonic solutions (e.g., salt, sugars) to patients with edema. This is due to a temporary transfer of excessively large quantities of fluid from the tissues to the blood stream; as a result of the usually prompt diuretic response, however, the plasma volume returns to normal or may actually decrease for a time if excessively large amounts of fluid are eliminated. A slight increase in blood volume has been reported occasionally in patients with essential hypertension, Raynaud's disease, thromboangiitis obliterans, hyperthyroidism, the nephrotic syndrome and congestive heart failure. However, these conditions are rarely accompanied by any significant deviation from the normal in the absence of complicating factors, the majority of which tend to lower rather than to raise the plasma volume.

Decreased Blood Volume. Diminution in blood volume may be due to a decrease in the volume of corpuscles or of plasma or both. Immediately after a severe hemorrhage the blood volume is decreased due to loss of both cells and plasma. The plasma volume is quickly restored, particularly if fluids are administered in adequate amount and, after the first twenty-four hours, the plasma volume may be actually greater than normal. However, the total blood volume is not restored for some time, depending upon the severity of the blood loss. This restored plasma is usually deficient in protein, the plasma protein concentration usually being restored to normal before the red blood cells and hemoglobin concentration. In the majority of chronic anemias the total blood volume is usually normal or slightly subnormal, the plasma volume being normal or increased. In pernicious anemia the total blood volume is low, the plasma volume being usually normal or subnormal. A decrease in total blood volume has also been observed in hemolytic jaundice, the plasma volume usually being somewhat increased. Elevated blood and plasma volumes have been reported in patients with splenic anemia and portal cirrhosis.

A marked decrease in circulating blood volume is a prominent feature of the shock syndrome, which may be due to a variety of causes. Except in the presence of hemorrhage, this decrease is usually due almost entirely to a decrease in plasma volume, contributed to by the transudation of fluid, and usually also some protein, through the abnormally permeable capillaries, and by sequestration of blood in large capillary beds (e.g., splanchnic). This results at times in a state of hemoconcentration, as evidenced by high hematocrit values and red blood cell counts. Subnormal total blood and plasma volumes, with hemoconcentration, may also be observed under the following conditions: (*a*) prolonged water restriction; (*b*) excessive water loss (diuresis, diarrhea, vomiting, intestinal fistula, pancreatic and biliary fistula, sweating); (*c*) acidosis, in which the loss of plasma water may be due in part to diuresis and in part to transfer of water from the plasma to the cells; (*d*) advanced diabetes mellitus, in which the low plasma volume may be due in part to diuresis and in part to acidosis; (*e*) excessively high external temperatures, in which large quantities of water may be lost through the skin and lungs; (*f*) occasionally in uremia, due to such complicating factors as acidosis, vomiting, diuresis, diarrhea, etc.; (*g*) ether anesthesia; (*h*) adrenal cortical insufficiency, in which the low plasma volume results mainly from the excessive loss of sodium and chloride ions, with water, in the urine, or their passage from the plasma into the tissues (p. 438). It should be emphasized that, with the possible exception of the shock syndrome and adrenal cortical insufficiency, marked decrease in plasma volume occurs only when the factors which operate to produce it act over long periods of time. As was suggested above, any tendency toward the loss of fluid from the blood plasma is usually promptly counteracted by the passage of fluid from the interstitial fluid compartment into the vascular compartment. Since the reserve supply of fluid in the interstitial tissues is about three times as great as the normal plasma volume, it is obvious that significant diminution in the latter occurs only usually with extensive depletion of the body fluids. Adrenal cortical insufficiency and clinical Addison's disease constitute exceptions to this rule because of the fact that in the absence of adequate amounts of cortical hormones the organism is unable to maintain the normal concentration of sodium ion in the plasma, its loss involving necessarily the simultaneous loss of considerable amounts of water (p. 281).

CHANGES IN INTERSTITIAL FLUID

Abnormal changes in the volume of interstitial fluid may be in the direction of either an increase or a decrease, the former constituting a state of edema and the latter a state of dehydration. The chemical composition of interstitial fluid is essentially the same as that of blood plasma, differing from the latter chiefly in that it contains much less protein, somewhat more chloride ion and less calcium and magnesium. It should be emphasized that, from the standpoint of osmotic equilibrium and acid-base balance, the most important constituents of the fluid, as of the blood, are sodium on the cation side and chloride and bicarbonate on the anion side. Moreover, the integrity of the chemical pattern of extracellular fluid (blood plasma and interstitial fluid) and the stability of its physicochemical properties depend largely upon the maintenance of normal quantities of sodium and chloride. Changes in bicarbonate, except those due to its excessive administration, are usually dependent upon primary changes in the amount of chloride ion or upon the presence of increased quantities of abnormal acids, such as the ketone acids. The relation of these elements to the hydrogen ion concentration and osmotic value of extracellular fluid are discussed elsewhere in detail (p. 300ff.), and may be summarized as follows:

(1) The two factors which immediately control the pH of extracellular fluid are the relative concentrations of carbonic acid and of bicarbonate, the normal ratio being approximately 1 : 20.

(2) Since the normal concentration of the former is maintained quite accurately by the respiratory mechanism, deviations from the normal pH (acidosis or alkalosis) are almost invariably caused clinically by changes in the concentration of bicarbonate ion.

(3) Changes in the latter almost always result from alteration in some other part of the electrolyte structure. For example, if the sum of the other acid radicals (Cl, PO_4, SO_4, organic acids) is increased, an equivalent amount of bicarbonate will be dispossessed of base and liberated as free carbonic acid (acidosis). On the other hand, if the sum of the other acid radicals is decreased, the base previously combined with these radicals is immediately covered by bicarbonate ion, which is then increased correspondingly (alkalosis). In other words, the value for bicarbonate in the plasma is determined by the extent to which fixed base exceeds the sum of the acid radicals other than bicarbonate.

(4) Obviously, therefore, the normal value for bicarbonate depends upon the integrity of the entire electrolyte structure, the most important components of which, from a quantitative standpoint, are sodium and chloride (Fig. 29).

(5) The osmotic value of extracellular fluid is, of course, determined by the total concentration of its chemical components, but chiefly that of the electrolytes.

(6) Since changes in the concentration of chloride and other anions (other than bicarbonate) are offset by reciprocal changes in the concentration of bicarbonate, as stated above, it follows that the total base concentration determines the total osmotic value of the electrolytes.

(7) Because sodium constitutes such a large portion of the base structure of extracellular fluid, the osmotic value of the latter rests almost entirely on this element.

Dehydration. From the biochemical standpoint, a discussion of dehydration, i.e., a decrease in the volume of extracellular and intracellular fluids, consists mainly in a discussion of its consequences, namely, the resulting changes in electrolyte and acid-base balance and in the electrolyte composition of the body fluids. Changes in the composition of the blood plasma may be regarded as indicative of changes in interstitial fluid under these circumstances. Typical abnormalities in the plasma electrolyte pattern in some of the common clinical disorders accompanied by dehydration are presented in Figure 30, which does not, however, include changes in potassium (p. 289).

Although dehydration, as encountered clinically, is due occasionally to rigid restriction of water intake, it usually results from excessive loss of one or more of the body secretions or excretions, as in: prolonged vomiting or diarrhea due to any cause; intestinal, pancreatic, or bile fistulae; prolonged tube drainage of stomach or intestine; excessive diuresis; excessive sweating. The effects of such loss upon the electrolyte pattern of the body fluids depend largely upon the composition of the fluid which is lost and upon associated abnormalities in renal function. The average electrolyte composition of the digestive fluids and sweat is indicated in Figure 31, and the composition of urine in Figure 53 (p. 564).

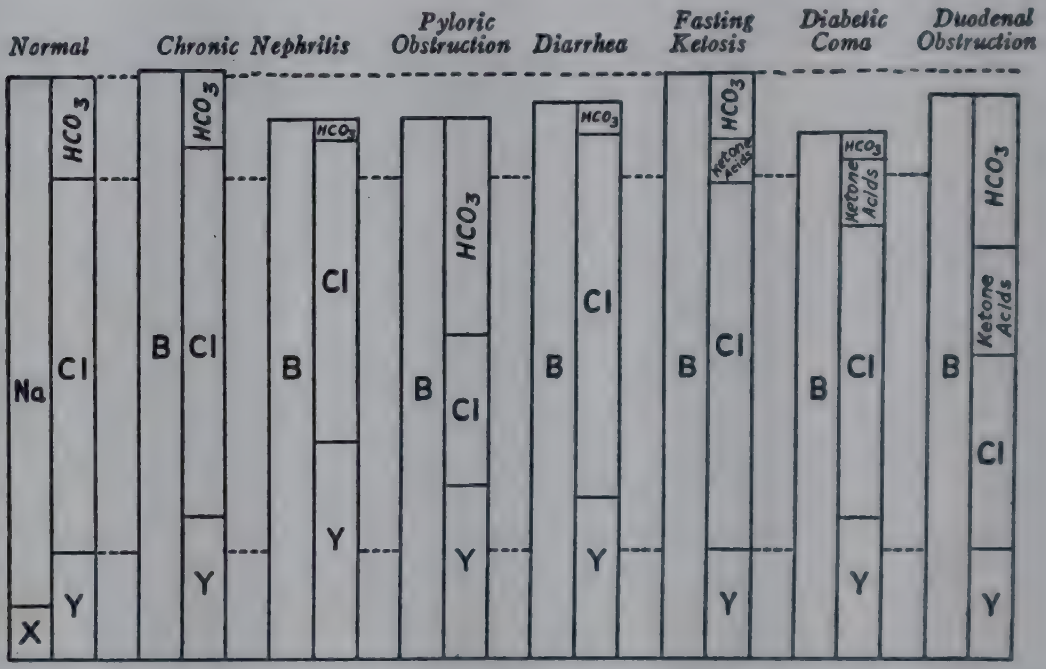

Fig. 30. Changes in the electrolyte pattern of the plasma in various disease conditions. Dotted lines indicate the normal levels of bicarbonate and chloride. B-total base; Y-unnamed acid radicals (after Gamble).

The changes which occur in electrolyte balance and distribution under these circumstances are considered in the discussions of disturbances of acid-base balance (p. 335 ff.) and of the metabolism of Na (p. 279), K (p. 287), and Cl (p. 279), and need not be reviewed here. However, certain generalizations may contribute to a better understanding of some of the fundamental effects of dehydration upon the organism.

1. Normal subjects lose about 500 cc. of water per sq. meter of body surface area per day (850 cc. daily by 70 kg. man) by vaporization from the lungs and skin ("insensible perspiration," p. 295). This is an obligatory loss, determined by metabolic activity (heat production) and is an important part of the mechanism of heat loss. If dehydration progresses to the point where this amount of water cannot be provided, the body temperature rises (dehydration fever). It has been estimated that this occurs when about 60 cc. of water per kg. body weight have been lost.

2. In normal adults on an average diet, about 500 cc. of water are required for adequate renal excretion of solid materials (p. 296). If dehydration progresses to the point where this amount cannot be provided, certain urinary constituents, mainly urea, will be retained in the body fluids (i.e., rise in blood urea N concentration).

3. There are indications that dehydration per se, and/or decrease in plasma Na and Cl concentrations, which occur commonly in dehydration, may have a deleterious effect on renal function. A possible associated increase in protein catabolism may contribute to the increase in blood NPN. Severe dehydration, with decrease in plasma water, may result in decreased glomerular filtration. This may be aggravated by the frequently associated fall in arterial blood pressure (shock syndrome). The altered electrolyte distribution may interfere with normal tubular reabsorptive functions, leading to aggravation of the Na, Cl, and water deficits.

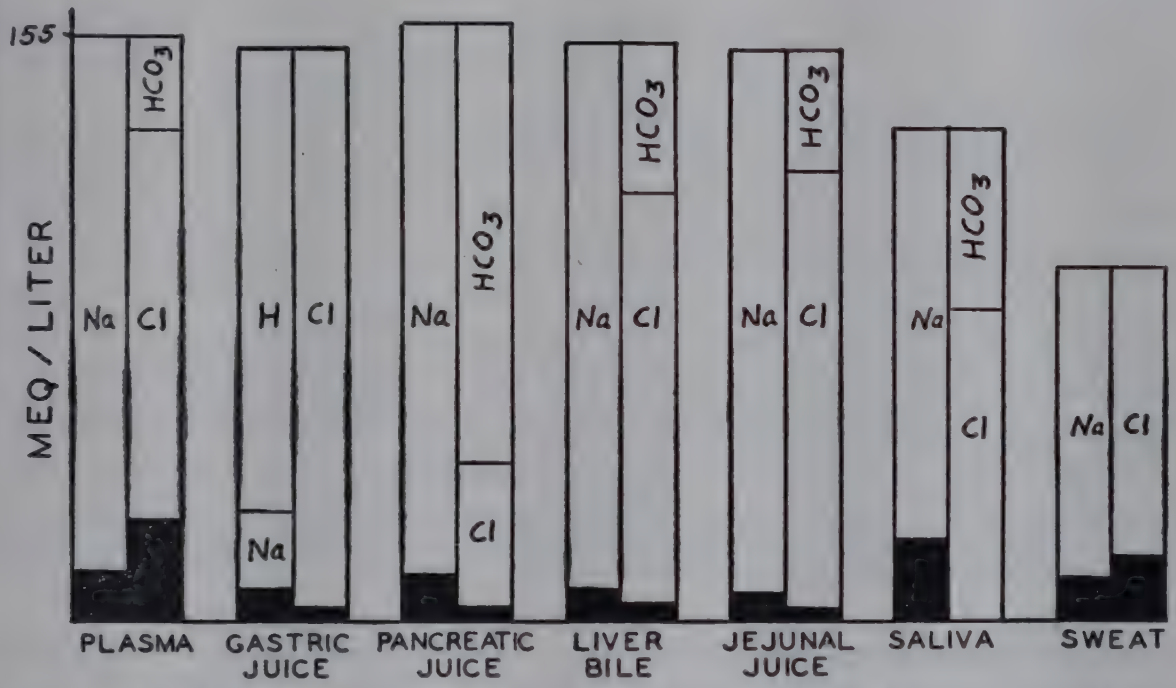

Fig. 31. The electrolyte composition of certain important body fluids, compared with that of blood plasma (Gamble).

4. Sodium chloride depletion has been produced in human subjects by means of a NaCl-free diet, ingestion of large quantities of distilled water, and the induction of profuse sweating through the medium of external heat. The chief objective phenomena are: (*a*) lowered plasma volume; (*b*) decreased concentrations of Na and Cl; (*c*) hemoconcentration (increased erythrocyte count and hemoglobin and protein concentrations); (*d*) increase in blood urea N; (*e*) fever.

5. Water depletion, without proportional NaCl depletion, occurs as a result of (*a*) rigid restriction of water intake, or (*b*) excessive perspiration in the absence of adequate water intake, sweat being a hypotonic fluid (Fig. 31, p. 313). Under these circumstances, not enough water being available to permit renal excretion of the Na and Cl now present in excess (i.e., in relation to water), the concentrations of Na and Cl in the plasma and other extracellular fluids increase (i.e.,

hypertonicity). This tends to draw water from the cells (intracellular dehydration and thirst).

6. Loss of fluid in dehydration is not restricted to the extracellular fluid compartment, as evidenced by urinary excretion of excessive amounts of potassium and phosphate in certain instances. These are excreted in such proportions as to indicate loss of intracellular fluid, in which they are present in much higher concentration than in extracellular fluids. Moreover, large amounts of potassium may be lost in gastrointestinal secretions in vomiting and diarrhea, being drawn ultimately from the intracellular fluid compartment in the absence of adequate intake of this element (p. 289). Recognition of the involvement of intracellular fluid (and potassium) as well as extracellular fluid (and sodium and chloride) in dehydration is of fundamental importance in connection with the treatment of these conditions (p. 281).

7. Ketosis may occur in the presence of alkalosis as well as acidosis (p. 95; Fig. 30, p. 312). For example, in prolonged vomiting (pyloric obstruction), ketosis may develop as a result of inadequate intake of carbohydrate.

8. The changes in acid-base balance that may occur as a result of loss of body fluids, e.g., gastrointestinal secretions, depend primarily upon the relative amounts of sodium and chloride ions which they contain. If Na is lost in excess of Cl (pancreatic, ileal, jejunal secretions; urine), there is a tendency toward acidosis; if Cl is lost in excess of Na (gastric juice), the tendency is toward alkalosis. This tendency toward alteration in the chemical structure of the extracellular fluid is influenced by the efficiency of renal function, the kidney being the chief organ of regulation of the composition of this fluid. Obviously, renal functional impairment, which occurs frequently as a consequence of dehydration (p. 591), results in further dislocation of the chemical structure of the extracellular fluids.

Increased Interstitial Fluid (Edema). Edema consists in the abnormal accumulation of interstitial fluid, either local or general. A detailed description of the factors involved in the pathogenesis of edema is beyond the scope of the present discussion. However, since the chemical composition of edema fluid is determined to some degree by the nature of the mechanism underlying its production, a brief consideration of these factors seems desirable.

The normal interchange of fluid between the vascular compartment and the tissue spaces depends primarily upon four factors: (1) the capillary blood pressure (13–35 mm. Hg), lower at the venous end than at the arterial end of the capillary, which tends to drive fluid toward the tissue spaces; (2) the colloid osmotic pressure (oncotic pressure) of the blood plasma (about 25 mm. Hg), dependent chiefly upon the concentration of plasma albumin (p. 146), which tends to draw fluid into the vascular compartment, counteracting the effect of the capillary blood pressure; (3) the relative impermeability of the capillary wall to protein; (4) the lymphatic circulation, which aids in the removal of fluid from the tissue spaces. A fifth factor is perhaps operative, namely, the resistance offered by the tissues themselves to the accumulation of fluid, usually termed the tissue tension, which varies in different parts of the body. Regardless of the nature of the clinical disorder in which edema may occur, the development of this phenomenon is almost invariably dependent upon abnormality in one or more of these fundamental factors. The various mechanisms responsible for the development of edema that operate at the capillary level may therefore be outlined as follows:

(1) *Increased Capillary Blood Pressure*
    (*a*) Congestive heart failure
    (*b*) Thrombophlebitis
    (*c*) External pressure on veins
    (*d*) Heat
    (*e*) Dependency
    (*f*) Vasodilatation (hemiplegia, trophedema)
(2) *Decreased Colloid Osmotic Pressure*
    (*a*) Loss of albumin
        (1) Urine
        (2) Ascites, etc.
    (*b*) Inadequate protein intake
        (1) Dietary restriction
        (2) Impaired absorption (vomiting, diarrhea, mucosal edema, etc.)
    (*c*) Impaired synthesis of plasma protein
        (1) Chronic infection
        (2) Anemia
        (3) Cachexia
        (4) Hepatic dysfunction
        (5) Nephritis
    (*d*) Sudden plasma dilution
        (1) Following sudden recovery from dehydration (diabetic coma, diarrhea in children, etc.)
        (2) Following acute massive hemorrhage
(3) *Increased Capillary Permeability* (allowing passage of protein and diminishing effective colloid osmotic pressure)
    (*a*) Inflammation (infection, burns, etc.)
    (*b*) Acute glomerulonephritis
    (*c*) Anemia
    (*d*) Congestive heart failure (anoxemia)
(4) *Decreased Lymphatic Drainage*
    (*a*) Lymphedema
    (*b*) Increased venous pressure (congestive heart failure)

Among important contributing factors the following may be mentioned: (*a*) a high intake of sodium if water is available, and vice versa; (*b*) low tissue tension, as in the case of the eyelids and genitalia, which favors the accumulation of fluid in those situations. Except under unusual circumstances, these contributory factors serve merely to increase the tendency to edema in the presence of one or more of the fundamental factors enumerated above.

ROLE OF HORMONES AND LIVER IN EDEMA. There is considerable evidence that edema in such common clinical disorders as congestive heart failure, cirrhosis of the liver, and renal disease (nephrotic syndrome), among others, is not due entirely to the factors outlined above, which act primarily upon the exchanges of water and solutes across the capillary wall. In these conditions there is apparently a fundamental defect in Na (and water) excretion, characterized by increased renal tubular reabsorption of Na (and also Cl and water), with consequent retention of abnormally large amounts in the body.

This abnormality in Na retention resembles that induced by certain steroid hormones, e.g., the "salt-active" adrenocorticoids (aldosterone, desoxycorticosterone) and, to a lesser degree, androgens, progesterone, and estrogens. The tendency of these hormones to produce edema (Na and water retention) is readily demonstrable experimentally, and is evidenced clinically in Cushing's syndrome (corticoids) and premenstrual edema (progesterone and estrogen). They probably act mainly on the renal tubule, stimulating reabsorption of Na, but other sites of action have not been excluded.

Because of these similarities, it has been suggested that Na retention in hepatic disease may be due, in part at least, to the action of excessive amounts of circulating "salt-active" steroid hormones, resulting from their inadequate removal from the systemic circulation ("inactivation") by the damaged liver cells (p. 539). Increased amounts of certain of these substances have been demonstrated in the blood and/or urine in such cases. The same hepatic mechanism may operate also in congestive heart failure, through the medium of reduced hepatic blood flow and hypoxia.

There have been reports of increased urinary excretion of an antidiuretic principle in patients with cirrhosis. Whether or not this is the posterior pituitary factor has not been established. Theoretically, excessive retention of Na might be expected to stimulate secretion of the antidiuretic principle by the pituitary, with consequent increased reabsorption of water by the renal tubule (p. 399). The hepatic depressor factor, VDM (apoferritin) (p. 550) may be involved, also; this substance produces antidiuresis under certain circumstances, presumably by stimulating the posterior pituitary to discharge the antidiuretic principle. There have been reports of increased amounts of VDM in the blood of patients with edema. This may be due to its inadequate destruction by damaged (or hypoxic) liver cells.

Role of Kidney in Edema. Sodium retention as a primary factor in the pathogenesis of edema is due fundamentally largely to increased Na reabsorption by the renal tubular epithelium. This may be due, in part at least, to the action of "salt-active" steroid hormones, VDM, and the pituitary antidiuretic principle, as indicated above. However, in congestive heart failure, hemodynamic influences may be operative also. Increase in renal venous pressure, with reduced renal blood flow, has been shown to result in increased tubular reabsorption of Na in acute, but not consistently in chronic experiments. In clinical states of congestive heart failure, this possible influence of renal venous stasis is supplemented by the associated hepatic congestion (reduced hepatic blood flow, hypoxia), with the consequences outlined above. It has been suggested, too, that excessive Na retention may contribute to the production of edema in the nephrotic syndrome, in addition to the more obvious factor of lowered plasma colloid osmotic pressure (hypoalbuminemia). The mechanism involved is not clear, but increase in antidiuretic hormone and "salt-active" corticoids has been suggested.

TRANSUDATES AND EXUDATES

The chemical examination of abnormal accumulations of fluid in the subcutaneous tissues and serous cavities frequently yields information of diagnostic importance. The chemical constituents of edema fluids are derived chiefly from the blood plasma, the composition of such fluids being dependent to some extent upon the permeability of the capillaries in the region involved, a factor which varies considerably in normal individuals in different vascular areas, and pathologically in response to abnormal stimuli. Alterations in the chemical composition of cerebrospinal fluid are considered elsewhere (p. 629). No attempt will be made to discuss in detail the chemical composition of edema fluid, attention being directed particularly to alterations which are of significance from the standpoint of diagnosis.

Specific Gravity. Crystalloids, being relatively readily diffusible, exist in practically the same concentration in edema fluids in different situations formed under similar conditions; such fluids, however, vary in specific gravity due to differences in their protein content, which are dependent upon variations in the permeability of the capillaries in different portions of the body. Normally the lymph coming from the lower extremities contains 2 to 3 per cent of protein, that coming from the intestines 4 to 6 per cent, while that from the liver contains 6 to 8 per cent. Similarly, it had been found that, in general, the quantity of protein in edema fluid in different localities varies in decreasing order as follows: (1) pleura, (2) peritoneum, (3) cerebrospinal, (4) subcutaneous. Some observers would vary this order slightly, reversing the positions of peritoneal and pleural effusions and of subcutaneous and cerebrospinal fluid. The statement is frequently made that the specific gravity of transudates is usually below 1.015 and that of exudates above 1.018; however, in view of the fact that the crystalloid content, with the exception of minor differences due to the existence of a Donnan equilibrium (p. 305), varies but slightly, the specific gravity varying more or less in direct proportion to the concentration of protein, there must be many exceptions to this rule since under identical conditions the protein concentration of edema fluids in various situations differs, as stated above. However, with these reservations in mind, statements made with regard to the difference in specific gravity of transudates and exudates may be accepted as a fairly satisfactory working basis for distinguishing between these two types of edema fluid. Values as high as 1.035 may be observed in inflammatory pleural and peritoneal exudates and values as low as 1.005 in subcutaneous transudate fluid.

Protein. Under normal conditions the fluid in the tissue spaces and in the serous cavities may be regarded as essentially protein-free filtrates of the blood plasma. The cerebrospinal fluid, which is the only one normally present in amounts large enough to enable quantitative analysis to be made with any degree of accuracy, normally contains 15 to 45 mg. of protein per 100 cc. If these figures may be accepted as representative of tissue fluids throughout the body, it is evident that their normal protein content is practically negligible as compared with that of blood plasma (6–8 gm. per 100 cc.).

The extremely low protein content of these fluids is due to the relatively poor diffusibility of protein through normal capillaries. Since inflammatory processes are associated with a marked increase in the permeability of the capillaries in the involved area, the degree of such increase being dependent upon the intensity of the process, the protein content of inflammatory exudates is relatively high. In purulent exudates resulting from a severe inflammatory process, as illustrated by empyema, the protein content of the serous portion of the fluid, obtained by centrifugation, may be approximately the same as that of the blood plasma. In the case of exudates resulting from inflammatory processes of lesser intensity, such as tuberculous pleurisy, tuberculous peritonitis, pneumonic pleurisy, meningitis, etc., the total protein concentration usually ranges from 0.1 to 5 gm. per 100 cc., being usually lower in the cerebrospinal fluid than in the peritoneal and pleural exudates. In contradistinction to the relatively high values observed in inflammatory fluids, the protein content of noninflammatory edema fluids or transudates is relatively extremely low since their pathogenesis is usually independent of alterations in capillary permeability. Thus the protein content of subcutaneous edema fluid is frequently below 0.1 gm. per 100 cc., that of pleural and peritoneal transudates occurring as a result of myocardial failure, nephrosis, uncomplicated cirrhosis of the liver, or the like being correspondingly low (0.1–1.0 gm. per 100 cc.). It must be realized, however, that, particularly in the case of pleural and peritoneal transudates, if the effusion has existed for some time, water may be reabsorbed more rapidly than solids, resulting in a slowly increasing concentration of protein which may eventually approach that of true exudates. It has been estimated that in the presence of a normal serum protein concentration, the presence of increased capillary permeability can be assumed with some degree of certainty only if the protein content of the edema fluid exceeds 4.1 gm. per 100 cc.

A relatively high protein content has frequently been found in the subcutaneous edema fluid of acute nephritis, suggesting its dependence upon generalized capillary injury rather than upon other forces which are believed to be responsible for the production of edema in chronic nephritis and myocardial failure. The protein concentration of blister fluid is also very high. Of particularly interest is the observation that the fluid of angioneurotic edema contains large amounts of protein, the inference being that this condition is associated with a marked increase in capillary permeability.

Albumin usually constitutes by far the largest part of the protein present in edema fluid, globulin occurring in small amounts and fibrinogen being seldom observed except in acute inflammatory exudates. The ability of these proteins to pass through the capillary wall is dependent upon the size of their molecules, those of greater size being more viscous and therefore passing with greater difficulty. The viscosity of plasma proteins varies in the following increasing order: albumin, pseudoglobulin, euglobulin and fibrinogen; therefore, in transudates, albumin may be present alone or may be accompanied by a small amount of pseudoglobulin; euglobulin and fibrinogen are usually present only in inflammatory exudates, the last named being indicative of an intense inflammatory reaction. Pneumococcus exudates appear to be particularly rich in fibrinogen, which is at times present in such high concentrations that the fluid may coagulate spontaneously.

In some cases a protein substance closely resembling mucin has been found in inflammatory exudates. Whereas the proteins mentioned above are believed to be derived from the blood plasma, this substance (mucin) appears to be a product of the inflamed cells. It has frequently been observed in joint fluids which, whether transudates or exudates, usually contain larger quantities of protein than similar fluids in other situations, the concentration of mucin being quite constant and apparently independent of the nature of the pathologic process.

Glucose. With the exception of cerebrospinal fluid, the fluid of the tissues and that of the pleura, peritoneum and other serous cavities contains sugar in practically the same concentration as that of the blood. The sugar present in these fluids appears to be chiefly glucose. Alterations in the glucose concentration of the blood are reflected in parallel changes in the glucose content of the various tissue fluids. The glucose content of pleural and peritoneal transudates is usually practically the same as that of the blood. The glucose content of inflammatory exudates is relatively low due to the destruction of glucose by the action of bacteria and cells present in the fluid, the degree of this reduction being dependent somewhat upon the intensity of the inflammatory process. The significance of this observation in the differential diagnosis of meningeal and cerebrospinal lesions is discussed in the section on cerebrospinal fluid (p. 631).

Chloride. The factors which determine the relationship between the chloride content of blood plasma and tissue fluid are discussed elsewhere (p. 305). The chloride content of noninflammatory edema fluid, or transudates, is higher than that of the blood plasma, ranging from 720 to 750 mg. per 100 cc. (expressed as NaCl), the difference being due to the existence of a Donnan equilibrium dependent upon the higher concentration of protein in the plasma as compared with normal tissue fluid and transudates. The chloride content of inflammatory exudates, which are relatively rich in protein, is lowered, approaching that of blood plasma, the degree of diminution varying roughly directly with the increase in the concentration of protein, in accordance with the laws governing the concentrations of readily diffusible substances on two sides of a semipermeable membrane under such circumstances (see p. 305). The chloride content of pleural effusions in pneumococcus pneumonia is particularly low because of the low chloride concentration of the blood plasma.

Lipid. Neutral fat and fatty acids are not usually present in transudates or inflammatory exudates. A small amount of lecithin, varying from 20 to 100 mg. per 100 cc., may practically always be demonstrated, existing party in the free state and partly in combination with protein. Cholesterol does not appear to be a constant constituent of transudate fluid but is practically always present in inflammatory exudates, particularly those of long standing, being probably derived in part from degenerative changes either in the cells present in the fluid or in those lining the serous sac or the abscess cavity. The cholesterol content of such long-standing effusions may decrease markedly following repeated tapping, values ranging from 1 to 4.5 gm. per 100 cc. having been observed to fall to 20 to 50 mg. following repeated aspiration. In some cases exhibiting high cholesterol values fat is also present, having been observed particularly in tuberculous pleural and peritoneal effusions. Capillaries appear to be permeable to cholesterol to about the same extent as to protein, this fact accounting for the very low cholesterol content of transudate fluids. This is true usually even in cases of the nephrotic syndrome, in which the concentration of cholesterol in the blood plasma may be enormously increased. In inflammatory exudates, associated with increased capillary permeability, the lipid content of the edema fluid roughly parallels its protein content.

Effusions which contain lipids in sufficient quantity to cause a milky appearance are designated chylous, chyliform and pseudochylous, depending upon their fat content and their pathogenesis. True chylous effusions are due to the escape of chyle from a ruptured or obstructed thoracic duct into the pleural or peritoneal spaces. The fat content of chylous fluid naturally varies with the quantity of fat ingested, being modified to a certain extent by processes of effusion or resorption occurring in the pleura or peritoneum. Values of from 0.05 to 3.85 gm. per 100 cc. have been reported. The concentration of cholesterol and lecithin, although increased, is usually low in proportion to that of neutral fat and fatty acids. Such fluids usually contain relatively high concentrations of protein, this factor also being largely dependent upon the composition of the diet, and spontaneous coagulation occurs not infrequently.

The term ‘‘chyliform effusion’’ is applied to fluids which may be identical in appearance with those described above, the fat content of which, however, is due not to chyle but to fatty degeneration of the cells present in the effusion or of those lining the walls of the cavity involved. Differentiation between chylous and chyliform effusions is frequently difficult since in both instances the turbidity is probably due to emulsified fat. However, in some cases the cholesterol and lecithin content of chyliform effusions is relatively higher and the fat content relatively lower than those of true chylous fluids. The term ‘‘pseudochylous effusion’’ is applied to fluids which may be turbid or milky in appearance but contain little or no fat, the turbidity being due chiefly to lecithin and cholesterol. It has also been demonstrated that albumin in a highly dispersed state may impart a milky appearance to such fluids. Subcutaneous pseudochylous fluid in the lower extremities and the scrotum may result from obstruction of lymphatic vessels by filaria. Similar fluids in the pleural and peritoneal cavities have been observed in lipoid nephrosis and in chronic glomerulonephritis with superimposed nephrotic lesions. They have also been observed in association with carcinoma of the peritoneum and in tuberculous pleurisy and peritonitis. Pseudochylous fluids may appear to be relatively clear when first removed, the turbidity and milky appearance increasing upon cooling. Relatively large amounts of protein may be present, varying from 0.1 to 4.2 gm. per 100 cc., and spontaneous coagulation, though not as commonly observed as in true chylous effusions, may nevertheless occur.

Other Constituents. Creatinine, uric acid, and particularly urea are present in exudates and transudates in practically the same concentrations as in the blood. Some observers have found that in nephritis edema fluid may at times contain more nonprotein nitrogen than the blood, an observation which may be of significance but the practical importance of which has not been demonstrated. The calcium content of transudates, ranging from 4.5 to 5.5 mg. per 100 cc. in the case of fluid with a low protein content, apparently represents the normal diffusible fraction of serum calcium. With increasing values for protein in both transudates and exudates the calcium concentration increases, the increase representing a nondiffusible fraction, which is probably in combination with protein. Transudates contain approximately the same concentration of inorganic phosphorus as the blood serum, and the concentration of bicarbonate is somewhat higher and that of sodium somewhat lower than in serum. The concentration of magnesium in transudate fluids averages about 65 per cent and that of potassium about 80 per cent of that in the serum. In the presence of increased protein concentration in the fluid, the concentration of magnesium increases.

Small amounts of bilirubin may be present in transudate fluid in the presence of hyperbilirubinemia. Larger amounts may be found in exudate fluids under such circumstances in concentrations varying roughly in proportion to the concentration of protein in the fluid. Bilirubin has been demonstrated in pleural and peritoneal effusions in patients with congestive heart failure and cirrhosis of the liver without hyperbilirubinemia (degradation of extravasated hemoglobin).

BIOCHEMICAL CHANGES IN SHOCK

Although the fundamental primary disturbance in shock is a serious reduction in venous return and, therefore, in the effective circulating blood volume, certain biochemical changes develop which contribute to the characteristic clinical picture.

The marked drop in blood volume occurs at the time of injury (or hemorrhage), the typical clinical picture of traumatic shock occurring after reduction of at least 30 per cent in plasma volume. Metabolic disturbances may appear when the reduction exceeds 20 per cent. The progressive character of shock apparently depends upon a steady decrease in cardiac output which, after the immediate large fall incident to the primary reduction in blood volume, declines gradually to very low levels. This is accompanied by a state of stagnant anoxia, with progressive decrease in oxygen consumption.

The blood sugar concentration rises in the early stages, due presumably to increased secretion of epinephrine. It falls subsequently to hypoglycemic levels, due apparently to increased utilization in the tissues. There is an associated increase in blood lactate and pyruvate, suggesting a shift from the aerobic to the anaerobic type of glycolysis (also hepatic functional impairment).

There is evidence of an increase in the rate of breakdown of protein in the peripheral tissues, and an increase in blood amino acids and lactic acid, due probably also to hepatic functional impairment. The liver is exceedingly vulnerable to oxygen deprivation. The blood NPN rises, due in part to increased protein catabolism, but chiefly to renal functional impairment. The primary fall in blood pressure in shock is followed by generalized arteriolar constriction, in which the renal arterioles participate. Inasmuch as about one-fourth of the resting cardiac output normally perfuses the kidneys, this renal vasoconstriction plays an important homeostatic role in shock. This circulatory homeostasis, however, is effected at the expense of renal function, both glomerular and tubular function being seriously impaired by the hypotension and renal ischemia. Renal blood flow and glomerular filtration diminish, with oliguria or anuria and impairment or loss of concentrating power.

According to some, in the presence of liver anoxia, excessive amounts of hepatic vasodepressor material (VDM) enter the blood stream (inadequate destruction). This, together with a simultaneous decrease in production of vasoexcitor material (VEM) by the anoxic kidney, results in excessive arteriolar dilatation, and is regarded by some as playing an important role in the pathogenesis of shock.

A severe acidosis develops, due largely to increasing retention of fixed acids (lactate, pyruvate, phosphate, sulfate), and accompanied by marked reduction in arterial CO_2 and plasma CO_2 combining power.

The concentration of sodium remains essentially unaltered. There is little or no change in serum potassium until shortly before death; it then increases sharply, at times to very high levels (40–50 mg. per 100 cc.). Progressive hemoconcentration, once considered as evidence of the occurrence of generalized capillary leakage in shock, is usually not observed except in cases of burns, peritonitis, or those complicated by infection, long exposure, and dehydration.

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APTER XV

"'Acid-Base'" (Anion-Cation) Balance (Neutrality Regulation)

rmal metabolic processes involve continual production of relatively large ounts of acid substances. The most important of these include carbonic (CO_2), furic, phosphoric, and organic acids, such as lactic and β-hydroxybutyric. CO_3 is the chief acid formed in the course of cellular oxidations, about 10 to or more mols being produced daily (equivalent to 1-2 liters of concentrated Cl). Sulfuric (oxidation of S of protein), phosphoric (oxidation of P of protein d lipids), and other acids are produced in quantities of about 80 to 120 milliols daily under ordinary conditions. Although foodstuffs provide a certain nount of alkaline substances, this is far exceeded by their potential acid content. he body is therefore faced with the necessity of continually removing large uantities of acid which is being formed within the cells. These substances must e transported to the organs of excretion in the extracellular fluids without nduly affecting their H^+ ion concentration, which is maintained at approximately H 7.4 and is one of the most rigidly controlled features of their electrolyte attern. The mechanisms by which these ends are accomplished comprise the echanism of regulation of "acid-base" balance. These may be outlined as llows:

1. Dilution.
2. Buffer systems; restriction of pH change in body fluids.
3. Respiration; ultimate excretion of CO_2.
4. Renal mechanism.
    (a) Excretion of excess "acid" or "base."
    (b) Ammonia formation; "base" conservation.

Use of the terms "acid" and "base" to indicate the anions and cations, reectively, of the body fluids is not acceptable from a chemical standpoint. An cid is defined as a substance (ion, molecule, particle) that yields H^+ ions (prons) in solution and a base is anything that combines with H^+ ions. Accordingly, hereas H_2CO_3 is an acid, dissociating into H^+ and HCO_3^- ions, its anionic mponent, HCO_3^- is a base. Other examples are:

Acid		Base
HSO_4^-	$\rightleftarrows H^+ +$	$SO_4^{=}$
CH_3COOH	$\rightleftarrows H^+ +$	CH_3COO^-
$H_2PO_4^-$	$\rightleftarrows H^+ +$	$HPO_4^{=}$

$NaHCO_3$ acts as a base because it yields HCO_3^- ions, which can combine with H^+ ions.

On this basis, the ionic structure of the body fluids should be regarded as consisting of anions and cations, rather than of "acids" and "base," and the term "anion-cation" balance should be substituted for "acid-base" balance. However, the latter designation has become firmly established in clinical usage and we feel that an attempt to eliminate it entirely from a text for clinicians at this time would lead to confusion. There is, in fact, some logical basis for employment of this inaccurate terminology. When an acid, e.g., acetic acid, is added to the essentially neutral (pH 7.4), buffered extracellular fluids, the H^+ ions of the acid largely disappear in the formation of a poorly dissociated molecule, viz., H_2O, and the pH of the fluid is only very slightly changed. The most obvious indication of the addition of the acid is the presence of an increased concentration of the acid anion, in this case acetate ion, which occurs at the expense of the buffer anions (HCO_3^-, $HPO_4^=$, protein). Inasmuch as the addition of the acid is reflected ultimately in the quantitative distribution of these anions, they have been referred to as the "acid" portion of the body fluid electrolytes. Conversely, the addition of alkalis, e.g., NaOH, will be reflected almost entirely in an increase in the cation Na^+, which has therefore been referred to as a "base." Although admittedly inaccurate, the designation "acid-base" balance is consequently employed synonymously with anion-cation balance.

PHYSIOLOGICAL BUFFER SYSTEMS

The capacity of the extracellular fluids for transporting acids from the site of their formation (cells) to the site of their excretion (e.g., lungs, kidneys) without undue change in pH is dependent chiefly upon the presence of efficient buffer systems in these fluids and in the erythrocytes. Each buffer system consists of a mixture of a weak acid, HA, and its salt, BA (p. 327). The most important of these are as follows:

$$\textit{Plasma:}\ \frac{H_2CO_3}{BHCO_3},\ \frac{H\cdot\text{protein}}{B\cdot\text{protein}},\ \frac{BH_2PO_4}{B_2HPO_4},\ \frac{H\cdot\text{organic acid}}{B\cdot\text{organic acid}}$$

$$\textit{Erythrocytes:}\ \frac{H_2CO_3}{BHCO_3},\ \frac{HHb}{BHb},\ \frac{HHbO_2}{BHbO_2},\ \frac{BH_2PO_4}{B_2HPO_4},\ \frac{H\cdot\text{organic acid}}{B\cdot\text{organic acid}}$$

The buffer systems in the interstitial fluids and lymph are much the same as in the blood plasma, except that proteins are generally present in much smaller quantities. The buffer systems in intracellular fluids are also qualitatively much the same as in the plasma, but the cell fluids contain much higher concentrations of protein.

Although all of these buffer systems are operative to a certain extent within the physiological range of pH values, only a few of them exist in sufficiently high concentrations to be of distinct quantitative significance in the regulation of acid-base balance. In the blood plasma, the bicarbonate and plasma protein systems, and in the erythrocytes, the bicarbonate and hemoglobin systems play the most important roles in this connection.

Buffer Action of Hemoglobin. The buffering capacity of Hb, as of any protein, depends upon the number of dissociated buffering groups, acidic (carboxyl) or basic (amino, guanidino, and imidazole), which varies with the pH of the medium. Within the pH range 7.0 to 7.8, most of the physiological buffering action of Hb is due to the imidazole groups of histidine.

The degree of dissociation of a buffering group in a protein, which determines its buffering capacity, is influenced by adjacent groups in the molecule. In the case of the imidazole group of histidine, which is intimately associated with the iron of hemoglobin, its strength as a buffer is affected by changes in the degree of oxygenation of hemoglobin. When oxygen is removed, the imidazole group is rendered less acidic, consequently less dissociated, removing a hydrogen ion from solution and becoming electrically positive. This effect is reversed with increased oxygenation of the hemoglobin molecule. This reaction indicates that not only is the buffering capacity of Hb related to the degree of oxygenation, but also that its ability to accept or liberate oxygen is influenced by the acidity of the medium. A decrease in acidity facilitates oxygenation of Hb and an increase in acidity facilitates liberation of oxygen, producing characteristic changes in the oxygen dissociation curve of Hb (p. 357).

The implications arising from these considerations may be summarized as follows:

1. Oxygenation of Hb increases its acidity, causing it to give up hydrogen ions to the medium, i.e., oxyhemoglobin is a stronger acid than reduced hemoglobin.
2. Reduction of oxyhemoglobin (i.e., deoxygenated Hb) decreases its acidity, removing hydrogen ions from the medium.
3. Introduction of an acid, e.g., H_2CO_3, into a medium containing oxyhemoglobin facilitates loss of oxygen, i.e., formation of reduced hemoglobin. The latter, being less acid (i.e., more basic) than oxyhemoglobin, is better able than the latter to counteract the acidifying effect of the added acid (p. 326).

The buffer mechanisms involved in the case of H_2CO_3, the anhydride of which (CO_2) is volatile, differ from those for the stronger, nonvolatile, fixed acids (sulfuric, phosphoric, lactic, etc.).

Buffer Systems for H_2CO_3 (CO_2). H_2CO_3 is buffered chiefly by the imidazole group of Hb in the erythrocytes and proteins in the plasma. Hb exerts by far the greater effect in this connection because its capacity for combination with H^+ ions greatly exceeds that of the plasma proteins. The over-all reactions may be indicated as follows:

(Plasma)

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

$$H_2CO_3 + \text{protein} \rightleftharpoons HCO_3^- + H^+ \cdot \text{protein}$$

(Erythrocytes)

$$CO_2 + H_2O \xrightleftharpoons[\text{anhydrase}]{\text{carbonic}} H_2CO_3$$

$$H_2CO_3 + Hb \rightleftharpoons HCO_3^- + H^+ \cdot Hb$$

$$H_2CO_3 + HbO_2 \rightleftharpoons HCO_3^- + H^+ \cdot HbO_2$$

The effect of addition of CO_2 is to increase both H_2CO_3 and $BHCO_3$, the latter owing to removal of additional H^+ ions by Hb and, to a minor extent, by the plasma proteins. The liberation of oxygen by Hb in the tissues, which occurs simultaneously with the addition of CO_2, increases the buffering capacity of the Hb, as indicated above. The reciprocal exchanges of Cl^- and HCO_3^- ions between the plasma and erythrocytes (chloride shift) constitute an additional important phase of the extremely efficient mechanism for buffering and transporting H_2CO_3 (p. 358) (Fig. 32, p. 327).

CO_2 ENTERS PLASMA IN TISSUES (p. 359). As indicated below, the largest portion of the added CO_2 diffuses into the erythrocytes. The remainder stays in solution of the plasma, reacting with water as follows:

$$CO_2 + H_2O \rightleftarrows H_2CO_3$$

This reaction occurs slowly; the equilibrium is far to the left, the concentration of dissolved CO_2 being about 1000 times that of H_2CO_3. The small increase in the latter which occurs when CO_2 is added to the plasma in the tissues ionizes ($H_2CO_3 \rightleftarrows H^+ + HCO_3^-$), the added H^+ ions being buffered by the relatively weak plasma buffer systems (viz., proteins). The pH falls slightly.

CO_2 ENTERS ERYTHROCYTES (p. 359). The bulk of the added CO_2 diffuses into the erythrocytes chiefly because the reaction, $CO_2 + H_2O \rightleftarrows H_2CO_3$, progresses very rapidly to the right by virtue of the catalytic action of the enzyme, carbonic anhydrase (C-A). A small amount of CO_2 remains as such in solution. Considerably more forms carbamino compounds with Hb ($\mathbf{R}$—$NH_2 + CO_2 \rightleftarrows \mathbf{R}$—$NHCOO^- + H^+$). The reaction is facilitated by the simultaneous liberation of oxygen, with an increment in reduced Hb, which is a weaker acid than oxyhemoglobin.

Most of the H_2CO_3 newly formed in the erythrocytes undergoes ionization ($H_2CO_3 \rightleftarrows H^+ + HCO_3^-$). This reaction proceeds to the right chiefly because both ions are removed rapidly, H^+ being buffered by Hb, and HCO_3^- diffusing into the plasma.

BUFFERING OF CO_2 BY HB. Reduced Hb, being a considerably weaker acid than oxyhemoglobin, has a higher H^+-binding capacity than the latter. At the erythrocyte pH (7.25), 1 mM. of HbO_2 yields 1.88 mEq. of H^+ ion, whereas 1 mM. of Hb yields only 1.28 mEq. Consequently, in the tissue capillaries, the liberation of each millimol of O_2 (22.4 ml.) permits binding of 0.6 mEq. more of H^+ ion, permitting the formation of 0.6 mM. of $BHCO_3$ from 0.6 mM. of CO_2 (13.4 ml.) with no change in pH. At a respiratory quotient of 0.6, i.e., $\frac{0.6 \text{ mM. } CO_2 \text{ produced}}{1.0 \text{ mM. } O_2 \text{ consumed}}$ (p. 364), 0.6 mM. of H^+ ions will be formed (from 0.6 mM. H_2CO_3), which will be completely buffered as a result of reduction of Hb. At the usual respiratory quotient of 0.82, therefore, only 0.82 minus 0.6, or 0.22 mM. of H^+ ions per mM. of O_2 consumed must be buffered by other means. Under average conditions of hemoglobin content and saturation and CO_2 transport, over 73 per cent of the total amount of CO_2 entering the blood in the tissues is handled in this manner.

CHLORIDE-BICARBONATE SHIFT. Because of the great rapidity with which hydration of CO_2 (transformation to H_2CO_3) occurs in the erythrocytes as com-

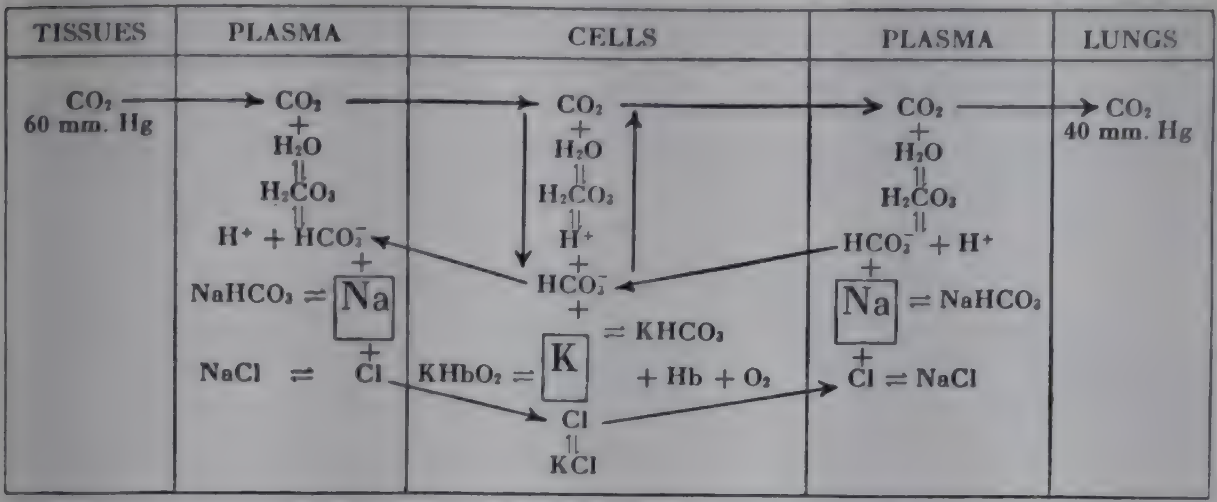

Fig. 32. Exchange of HCO_3^- and Cl^- between the red blood cells and the plasma in the tissues and the lungs (chloride shift). The heavy arrows indicate the direction of the changes in these situations.

pared with the plasma, the concentration of HCO_3^- rises in the former faster than in the latter. Inasmuch as the erythrocyte wall is permeable to HCO_3^-, this ion diffuses from the erythrocytes into the plasma. According to the laws of membrane equilibrium, the ratios of the concentration of individual diffusible monovalent ions in the cells and plasma must be equal, i.e.,

$$\frac{[HCO_3^-] \text{ cells}}{[HCO_3^-] \text{ plasma}} = \frac{[Cl^-] \text{ cells}}{[Cl^-] \text{ plasma}}$$

Consequently, as HCO_3^- passes from the erythrocytes into the plasma, Cl^- passes in the opposite direction. In order to preserve electrical neutrality of the cell and plasma fluids, the numbers of negative ions (i.e., Cl^- and HCO_3^-) exchanged must be identical. The Cl^- ions passing into the erythrocytes are balanced electrically by the K^+ ions previously balanced by the HCO_3^-, while the latter ions entering the plasma are balanced by the Na^+ ions previously balanced by the diffused Cl^-. As a result of these changes, most of the HCO_3^-, although formed originally in the erythrocytes, is actually transported in the plasma (Table 20, p. 359).

All of the above reactions are reversed in the lungs, the quantity of CO_2 entering the blood in the tissues passing into the alveoli and the quantity of oxygen lost in the tissues being restored from the alveolar air.

Buffer Systems for Fixed Acids. Neutralization of fixed (nonvolatile) acids entering the extracellular fluids is accomplished chiefly by the $H_2CO_3/BHCO_3$ buffer system. Such acids (e.g., HCl, H_2SO_4, lactic, etc.) react with $BHCO_3$ as follows:

$$HCO_3^- \text{ (from } NaHCO_3) + H^+ \text{ (from HCl)} \rightleftharpoons H_2CO_3$$

Protein and phosphate buffer systems may also be involved, but to a relatively minor extent.

$$\text{Protein} + H^+ \rightleftharpoons H^+ \cdot \text{Protein}$$

$$HPO_4^- + H^+ \rightleftharpoons H_2PO_4^-$$

Bicarbonate is particularly efficient in this connection because (1) it is present in higher concentration than the other buffer salts, and (2) the acid product of the reaction, H_2CO_3, is effectively buffered (p. 326) and is readily disposed of by the lungs (p. 354) by virtue of the volatility of its anhydride, CO_2, rendering the reaction irreversible.

$$H_2CO_3 \rightleftharpoons H_2O + \overset{\nearrow}{CO_2} \quad \text{(expired air)}$$

If a fixed base (e.g., NaOH) enters the extracellular fluid, it reacts with the acid components of these buffer systems, chiefly H_2CO_3:

$$\begin{gathered} H_2CO_3 + OH^- \text{ (from NaOH)} \rightleftharpoons Na^+HCO_3^- + H_2O \\ H^+ \cdot \text{Protein} + OH^- \rightleftharpoons \text{Protein} + H_2O \\ H_2PO_4^- + OH^- \rightleftharpoons HPO_4^= + H_2O \end{gathered}$$

Net Effect of Buffer Mechanisms. Because of the relative abundance of bicarbonate in extracellular fluids, the chief effect of the entrance of metabolic acids, both carbonic and fixed acids, is to increase the concentration of H_2CO_3. In the case of the fixed acids, this is done at the expense of $BHCO_3$. In either case, the ratio, $H_2CO_3/BHCO_3$, which largely determines the pH of these fluids (p. 333), is increased, with a consequent increase in H^+ ion concentration. Under normal conditions this increase is but slight because (1) H_2CO_3, a weak acid (i.e., weakly dissociated), forms relatively few H^+ ions, (2) it is effectively buffered (p. 326), and (3) it is readily eliminated by the lungs as CO_2, the increase in concentration of both H_2CO_3 and H^+ being thereby minimized.

$$\overset{\nwarrow}{CO_2} + H_2O \leftarrow H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

The carbonic acid produced in the course of oxidative metabolic processes is thus effectively disposed of, chiefly by the hemoglobin mechanism, with very little change in pH (p. 326). The fixed acids can be buffered efficiently by bicarbonate as long as adequate amounts of the latter are present in the extracellular fluids. Because of this fact, and because the other buffer systems are in equilibrium with the $H_2CO_3/BHCO_3$ system, the blood bicarbonate, representing the extracellular fluid bicarbonate, is a measure of the alkali available for neutralization of fixed acids, and has been termed the "alkali reserve."

Respiratory Regulation of Acid-Base Balance. Participation of the respiratory mechanism in the regulation of acid-base balance is dependent upon (1) the sensitivity of the respiratory center (medulla) to very slight changes in pH and in pCO_2, and (2) the ready diffusibility of CO_2 from the blood, across the pulmonary alveolar membrane, into the alveolar air (p. 358).

An increase in blood pCO_2 of only 1.5 mm. Hg (0.2% increase in CO_2) results in a 100 per cent increase in pulmonary ventilation (stimulation of respiratory center), which increases also with slight increases in H^+ ion concentration of the blood. The excess CO_2 is thereby promptly removed from the extracellular fluids in the expired air. Decrease in blood pCO_2 or H^+ ion concentration causes depression of activity of the respiratory center, with consequent slow, shallow respiration, hypoventilation, and retention of CO_2 in the blood until the normal pCO_2 and pH are restored. This respiratory mechanism therefore tends to maintain the normal $H_2CO_3/BHCO_3$ ratio in the extracellular fluids in the face of the continual addition of H_2CO_3 as a result of the metabolic production of both CO_2 and fixed acids. According to the Henderson-Hasselbalch equation (p. 333), at pH 7.4, the $H_2CO_3/BHCO_3$ ratio is 1 : 20, which conforms to the observed concentration ratios of these substances in normal plasma, viz., 1.35 mEq. H_2CO_3/27 mEq. $BHCO_3$ per liter (3 vol. % H_2CO_3/60 vol. % $BHCO_3$).

Renal Regulation of Acid-Base Balance. Under ordinary dietary conditions, the amount of fixed acid produced daily exceeds the intake of available base by about 50 to 100 mEq. As indicated above, these acids are effectively buffered, at the expense, however, of a decrease in $BHCO_3$, the chief component of the buffer base available for this purpose (total about 1000 mEq.). The "alkali reserve" would eventually be exhausted if the increment in fixed acids (H^+ ions), temporarily neutralized by HCO_3^- anions, were not removed from the body and the $BHCO_3$ restored. This is accomplished by the kidneys, which are, consequently, the ultimate regulators of the acid-base balance, providing the most important final defense of the pH of the body fluids.

In the course of urine formation, a protein-free filtrate of blood plasma passes through the glomerular capillaries, the final composition of the excreted urine resulting from subsequent changes in this filtrate in the tubule (p. 564ff.). In normal subjects, under ordinary dietary conditions, the urinary pH is about 6.0. The difference in titratable acidity between the pH of the urine and that of plasma (7.4) represents the amount of acid removed by renal action from the plasma. Under conditions of extreme requirement for removal of excess acid or alkali respectively, the H^+ ion concentration of the urine can vary from pH 4.5 to 8.2 if the kidneys are functioning normally. This ability to excrete urine of variable acidity (or alkalinity) removes from the blood the quantity of excess acid or alkali required to preserve the normal H^+ ion concentration of the body fluids.

RENAL EXCRETION OF ACID. Even at the maximal attainable urinary acidity, pH 4.5, all $SO_4^=$ and Cl^- anions must be electrically balanced by B^+ cations, chiefly Na^+ and K^+, i.e., they cannot exist as free acids (sulfuric, hydrochloric). The same is true of over 90 per cent of the lactate anions. Consequently, at any urinary pH, these anions remove from the body practically the same amount of B^+ ("base") as is required to balance them electrically during their transport in the blood plasma; no significant deviation from the pH of the plasma (7.4) could be effected inasmuch as no H^+ ions could be carried out by these strong acid anions ($SO_4^=$ and Cl^-).

Increase in urinary acidity is accomplished by excretion of increased amounts of the weakly acidic BH_2PO_4 and certain weak organic acids, e.g., β-hydroxybutyric, acetoacetic, citric. In the plasma, at pH 7.4, phosphate exists as a mixture of about 80 per cent B_2HPO_4 and 20 per cent BH_2PO_4 ($Na_2^{++}\ HPO_4^=/Na^+\ H_2PO_4^- = 4/1$). At pH 4.8, about 99 per cent is in the form of BH_2PO_4 ($Na_2^{++}\ HPO_4^=/Na^+H_2PO_4 = 1/99$). According to these ratios, every five phosphate molecules circulating in the plasma and entering the glomerular filtrate carry nine negative charges, which must be balanced by nine positive charges, i.e., nine Na^+ ions. In the urine at pH 4.8, these five phosphate molecules, being virtually all in the form of $B^+H_2PO_4^-$, carry only slightly more than five negative charges and require only about five Na^+ ions for their neutralization. This degree of acidification of the urine, accomplished, as indicated below, by the addition of H^+ ions from the tubular cells, effects a saving of about four Na^+ ions for every five phosphate molecules excreted in the urine, the Na^+ being returned to the blood plasma.

A similar situation obtains with respect to weak organic acids, such as β-hydroxybutyric, acetoacetic, and citric. Anions of these acids must be transported in the plasma, at pH 7.4, completely covered by Na^+ cations, but a considerable portion can exist as the free acids in urine at pH 4.8. For example, at pH 4.8, about 45 per cent of the β-hydroxybutyrate anion in the urine exists as free acid and, consequently, excretion of each mol of this anion at this urinary pH releases and restores to the blood 0.45 mol of the Na^+ with which it had been carried in the plasma.

The process of acidification of the urine appears to be explicable most plausibly by the ionic exchange theory, although other explanations have been advanced. According to this view, CO_2 formed in the cells of the distal portion of the tubule (where acidification of the urine occurs), under the influence of carbonic anhydrase, is rapidly transformed to H_2CO_3, which undergoes ionization:

$$\text{CO}_2\ (\text{metabolic}) + \text{H}_2\text{O} \xrightleftharpoons[\text{anhydrase}]{\text{carbonic}} \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$$

The H^+ ions thus formed are passed into the lumen of the tubule, an equal number of Na^+ ions passing in the opposite direction into the cells (Fig. 33). Thus, a number of Na^+ ions, previously balancing $HPO_4^=$, citrate⁻, β-hydroxybutyrate⁻, etc., ions in the glomerular filtrate, are exchanged for H^+ ions.

$$\text{Na}_2^{++}\ \text{HPO}_4^= + \text{H}^+ \rightarrow \text{Na}^+\ \text{H}_2\text{PO}_4^- + \text{Na}^+$$
$$\text{Na}^+\ \beta\text{-hydroxybutyrate}^- + \text{H}^+ \rightarrow \beta\text{-hydroxybutyric acid} + \text{Na}^+.$$

The Na^+ ions, entering the cell, are absorbed into the blood stream with HCO_3^- ions formed from the H_2CO_3, thereby supporting the bicarbonate structure of the extracellular fluids (Fig. 33).

A portion of the urinary potassium enters the uriniferous tubule by active excretion by the distal tubular epithelium, in addition to the larger amount which filters through the glomeruli (p. 567). In this region (distal tubule), K^+ and H^+ ions apparently compete for some component of a common excretory mechanism, either ion being exchanged for Na^+ (Fig. 33). With increased concentration of K^+ in the renal tubular cells, more K^+ than H^+ ions are exchanged for Na^+, the acidity of the urine falls and that of the body fluids increases. Conversely, when the tissue cells (including kidney) are depleted of K^+, more H^+ and fewer K^+ ions are exchanged for Na^+, and the urine becomes more acid even though a state of alkalosis may exist. Inhibition of renal carbonic anhydrase results not only in ability to acidify the urine but also in striking increase in excretion of potassium, another indication of the competition between these two ions for tubular excretion (p. 567).

With increasing acidification of the glomerular filtrate, progressively more HCO_3^- is converted to H_2CO_3 and, consequently, to H_2O and CO_2, the latter diffusing across the tubule into the blood, the pCO_2 of which is in equilibrium with that of the fluid in the uriniferous tubule. This decrease in bicarbonate content of the urine, which may fall to zero as the acidity increases, is accompanied by a simultaneous increase in chloride. Changes in the reverse directions occur in the concentrations of these substances in the blood.

The phenomena described above occur in response to a stimulus provided by the kidney by an increase in H^+ ion concentration of the plasma and glomerular filtrate (increased requirement for excretion of acid and reabsorption of bicarbonate). If the plasma pH rises (alkalosis), the renal mechanism is stimulated in the opposite direction, i.e., decreased excretion of acid, decreased reabsorption of bicarbonate. The urinary acidity falls as a result of decreased exchange of H^+ and Na^+ ions, increasing amounts of phosphate being excreted in the form of Na_2HPO_4 and of organic acids as sodium salts. With increasing pH, up to 8.2, there is increased urinary excretion of HCO_3^- and of Na^+, the cation with which it is chiefly associated, excretion of Cl^- decreasing simultaneously. Changes in the reverse direction occur in the concentrations of these substances in the blood, tending to restore the normal pH of the plasma.

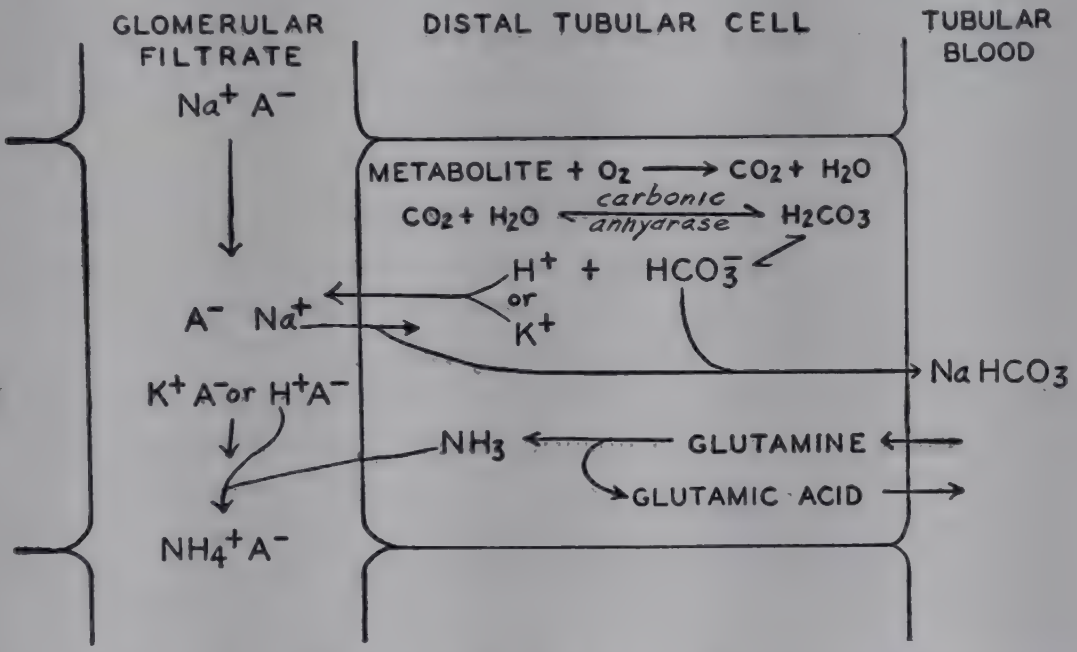

Fig. 33. Mechanism of acidification of urine and of ammonia formation and excretion by the kidney, indicating exchange of H^+ and/or K^+ for Na^+ (after Pitts).

Excretion of Ammonia. A normal subject, under ordinary dietary conditions, excretes 30 to 50 mEq. of ammonia daily. If the plasma H^+ ion concentration rises, the urinary ammonia increases, even tenfold in severe diabetic acidosis. A decrease occurs in alkalosis. The urinary NH_3 is formed in the cells of the distal portion of the uriniferous tubule, the site of acidification of the urine. About 60 per cent is derived from the amide group of glutamine (by the enzyme glutaminase) and the remaining 40 per cent from the α-amino group of certain amino acids (p. 151). Adrenal cortical hormones may perhaps exert a stimulating influence on this deamination process and, therefore, on NH_3 formation.

The NH_3 (gas) diffuses into the lumen of the tubule, combining with the H^+ ions in the acidified fluid to form NH_4^+ ions (Fig. 33). This effectively prevents undue accumulation of H^+ ions in the fluid, and therefore permits continued exchange of H^+ for Na^+ ions. The amount of Na^+ ion absorbed in the distal tubule is consequently reflected in the amount of both H^+ and NH_4^+ ions in the urine.

The urinary NH_3 begins to increase shortly after H^+ ion concentration of the blood rises, but the increase continues for some time after the blood and urine pH and bicarbonate content have reached their lowest level. The stimulus to NH_3 formation by the kidney is apparently similar to that producing acidification of the urine, i.e., decrease in pH and bicarbonate of the blood and glomerular filtrate. However, the duration of application of this stimulus influences NH_3 formation but not acidification of the urine.

ABNORMALITIES OF "ACID-BASE" (ANION-CATION) BALANCE

It is obvious from the preceding discussion that a number of interrelated factors and mechanisms are involved in the maintenance of the normal electrolyte pattern and pH of the body fluids, commonly referred to as "acid-base" balance, but more properly designated "anion-cation" balance (p. 323). Several of these do not lend themselves to direct quantitative study. However, factors operating in the blood can be investigated more or less readily because of accessibility of this medium. The most important of these are:

1. *Total "base"* (B^+): the sum of the concentrations (in mEq./liter) of all cations.
2. *H_2CO_3 content:* the concentration of H_2CO_3 (in mM./liter), which is in equilibrium with the pCO_2 (CO_2 tension) [pCO_2(in mm. Hg) $\times 0.3 = H_2CO_3$ (in mM./liter)].
3. *CO_2 content:* the concentration (in mM./liter) of total CO_2 in solution (HCO_3 plus H_2CO_3).
4. *HCO_3^- content:* the concentration (in mEq./liter) of HCO_3^-, usually calculated as the difference between the CO_2 and H_2CO_3 contents (thus including the carbamino-CO_2).
5. *CO_2 capacity or "combining power":* the HCO_3^- (or CO_2) content of blood or plasma saturated with 5.5 per cent CO_2 at 25° C.
6. *pH:* the logarithm of the reciprocal of the H^+ ion concentration.
7. *"Buffer-base":* the "base" equivalent to the sum of the concentrations (in mEq./liter) of buffer anions (whole blood = HCO_3^- plus Hb and plasma proteins; plasma = HCO_3^- plus plasma proteins).

The important role of Hb as a buffer has been pointed out elsewhere (p. 326), as has the difference in this connection between oxyhemoglobin and reduced hemoglobin. For most precise analysis of the state of "acid-base" balance, the following factors must be determined: (1) hematocrit (relative proportions of erythrocytes and plasma); (2) pH; (3) CO_2 content; (4) buffer base; (5) H_2CO_3 content (calculated from pCO_2 of blood or alveolar air). However, for practical clinical purposes, certain of these may usually be dispensed with (viz., hematocrit, buffer base) inasmuch as significant abnormalities due to changes in hemoglobin are reflected in the pH and HCO_3^- content.

As indicated below, clinical aberrations of "acid-base" balance are classified conveniently as primary disturbances in either HCO_3^- (bicarbonate) or H_2CO_3, the relation of which to the pH is expressed by the Henderson-Hasselbalch equation: $\text{pH} = \text{pK} + \log \frac{HCO_3^-}{H_2CO_3}$. At pH 7.4, the $HCO_3^- : H_2CO_3$ ratio is 20 : 1 (27 mEq. : 1.35 mEq./liter of plasma water). Knowledge of any two of these three factors (pH, HCO_3^-, H_2CO_3) therefore permits derivation of the third. Determination of the total CO_2 content (HCO_3^- plus H_2CO_3) and any one of the three factors mentioned permits derivation of the other two. Consequently, the most widely employed approach to the investigation of clinical disturbances of "acid-base" balance centers about these factors, perhaps the most useful for practical purposes being the simultaneous determination of pH (plasma or serum) and CO_2 content (plasma, serum, or whole blood).

Primary change in HCO_3^- (metabolic acidosis and alkalosis, p. 336) is by far the most common cause of acidosis and alkalosis of clinically significant degree. For this reason, reliance is frequently placed on determination of this factor alone for the diagnosis of acidosis (decreased HCO_3^-) and alkalosis (increase). The term "alkali reserve" has been applied to the plasma bicarbonate because of its important buffer action when fixed (nonvolatile) acids or bases are added to or removed in excess from the body fluids. Determination of the "CO_2 combining power (CO_2 capacity)" is often employed as a substitute for the HCO_3^- or CO_2 contents. It must be noted, however, that disturbances due to primary changes in H_2CO_3 (respiratory acidosis and alkalosis) are accompanied by alterations (secondary, compensatory) in HCO_3^- and CO_2 content in directions opposite to those which occur in "metabolic" acidosis and alkalosis (p. 335 ff.; Table 16). Unless the true nature of the disturbance is recognized, which is often possible (e.g., diabetes mellitus, nephritis, vomiting, diarrhea, hyperventilation, etc.), an incorrect diagnosis may be made on the basis of determination of this isolated factor.

Acidosis

The term "acidosis" is applied to the condition resulting from the formation or absorption of acids at a rate exceeding that of their neutralization or elimination. It may also, although much less frequently, be due to the loss of excessive quantities of base from the body. On the basis of the equation,

$$\text{cH} = \text{k} \frac{H_2CO_3}{BHCO_3},$$

it is evident that an increase in the hydrogen ion concentration of the blood (acidosis) may be caused by either a primary increase in the concentration of H_2CO_3 or a primary decrease in the concentration of $BHCO_3$. If these changes are of such magnitude that the hydrogen ion concentration rises above the upper limit of normal (pH below 7.3) the condition is one of uncompensated acidosis. However, as has been indicated above, because of the existence of a remarkably efficient compensatory mechanism, if the concentration of carbonic acid rises, the concentration of blood bicarbonate also tends to increase in order to maintain the normal equilibrium; likewise, as the concentration of blood bicarbonate diminishes, increased quantities of carbon dioxide are removed through the lungs with a consequent compensatory decrease in the concentration of carbonic acid in the blood. Because of these compensating processes, primary alterations in either of these two factors are, for a time at least, balanced to a certain degree by secondary changes in the other factor, as a result of which there may be little or no perceptible alteration in the hydrogen ion concentration. If under such circumstances the hydrogen ion concentration of the blood is maintained below the upper limit of normal (pH above 7.3) the organism is in a state of compensated acidosis. The clinical conditions in which a state of acidosis is commonly observed will be considered under two headings: (1) those associated with a primary increase in the concentration of H_2CO_3 in the blood; (2) those associated with a primary decrease in blood bicarbonate (alkali reserve).

Primary H_2CO_3 Excess (Respiratory Acidosis). Increase in the carbonic acid content (CO_2 tension) of the blood may occur in one of two general ways:

(1) Rebreathing, or breathing air containing abnormally high percentages of CO_2.

(2) Conditions in which the elimination of CO_2 through the lungs is retarded. In this group may be placed conditions causing mechanical asphyxia, morphine narcosis with its associated diminished minute ventilation, pneumonia (particularly bronchopneumonia), pulmonary emphysema and pulmonary fibrosis, in which the diffusion of CO_2 is impaired, and cardiac decompensation, in which pulmonary congestion and slowed circulation combine to cause increased CO_2 tension in the blood.

Compensatory Mechanisms. As the carbonic acid content of the blood increases, certain compensatory mechanisms come into play by means of which the organism attempts to maintain the hydrogen ion concentration of the blood within normal limits.

(1) *Increased Ventilation.* The increased stimulation to the respiratory center caused by the increased CO_2 tension of the blood results in increased depth and rate of respiration with consequent increased ventilation. As stated above, the sensitivity of the respiratory center to a relatively slight increase in hydrogen ion concentration or CO_2 tension of the blood renders this mechanism extremely efficient in counteracting the effects of primary CO_2 excess. This mechanism cannot of course be invoked in conditions in which the fundamental defect lies in depressed activity of the respiratory center, e.g., in morphine narcosis.

(2) *Increase in Bicarbonate.* In conditions associated with primary CO_2

excess the blood bicarbonate has been found to be increased. This simultaneous change in both elements of the bicarbonate system tends to limit the increase in hydrogen ion concentration of the blood which would otherwise occur. The increase in blood bicarbonate is accomplished by stimulation (by the increasing acidity) of the renal mechanism (p. 329), with consequent increased reabsorption of Na and HCO_3 and increased urinary acidity and ammonia.

(3) *Increased Ammonia Formation.* An increase in the rate of ammonia formation and excretion by the kidney tends to diminish the loss of base from the body (p. 329).

(4) *Increased Urinary Acidity.* The increasing acidity of the blood plasma stimulates the Na-H exchange in the distal portion of the renal tubules (p. 329), with consequent increase in urinary acidity.

Because of the efficiency of these compensatory mechanisms, primary CO_2 excess seldom results in a state of severe uncompensated acidosis as evidenced by the hydrogen ion concentration of the blood. From this standpoint, therefore, conditions in this group are of relatively minor importance clinically, symptoms of acidosis, apart from dyspnea, being encountered only infrequently and disturbance of the acid-base balance being seldom of sufficient magnitude to be of serious import.

BIOCHEMICAL CHARACTERISTICS (Table 16). Acidosis of this type is commonly designated "respiratory acidosis," for obvious reasons. If uncompensated, it is characterized biochemically (plasma or blood) as follows: (1) disproportionate increase in $[H_2CO_3]$ (pCO_2); (2) increase in $[HCO_3^-]$; (3) increase in CO_2 content; (4) decrease in $[HCO_3^-] : [H_2CO_3]$ ratio; (5) decrease in pH. If fully compensated, the CO_2 content is high, but the increase in $[H_2CO_3]$ and $[HCO_3^-]$ are proportionate, the $[HCO_3^-] : (H_2CO_3]$ ratio and pH remaining within normal limits. The urinary ammonia and titratable acidity are increased (if kidneys are functioning normally).

Primary Alkali Deficit (Metabolic Acidosis). Primary alkali deficit is the most common cause of acidosis occurring in clinical conditions. In this group may be placed the following conditions:

DIABETES MELLITUS (see p. 75). Acidosis due to alkali deficit is a constant feature of advanced diabetes mellitus. It is due largely to the existing ketosis and in part to the excessive loss of water with the associated elimination of excessively large quantities of base. The polyuria which attends the development of glycosuria is accompanied by a pronounced increase in the excretion of electrolytes, particularly sodium, potassium and chloride. This initial electrolyte loss is supplemented by a secondary increase in the loss of water and electrolytes simultaneously with the development of ketosis. These changes eventually lead to dehydration and depletion of body base.

The mechanism of production of ketosis in diabetes mellitus is discussed in detail elsewhere (p. 75). This results from excessive production of ketones in the liver because of inadequate carbohydrate utilization in the liver. The ketone substances, including aceto-acetic acid, beta-hydroxybutyric acid, and acetone,

*Table 16. Biochemical Characteristics of Uncompensated and Compensated Acidosis and Alkalosis**

PLASMA	NORMAL	ACIDOSIS				ALKALOSIS			
		HCO_3^- DEFICIT		H_2CO_3 EXCESS		HCO_3^- EXCESS		H_2CO_3 DEFICIT	
		Uncomp.	Comp.	Uncomp.	Comp.	Uncomp.	Comp.	Uncomp.	Comp.
HCO_3^-	mM./liter 23–28 (26) vol. % 51–62 (53.4)	– –	–	+	+	++	+	–	–
CO_2 capacity	mM./liter 24–35 (30) vol. % 53–78 (65)	– –	–	+	+	++	+	–	–
H_2CO_3	mM./liter 1.1–1.5 (1.3) vol. % 2.4–3.3 (2.9)	–	–	++	+	+	+	– –	–
pCO_2	mm. Hg 35–45 (40)	–	–	++	+	+	+	– –	–
CO_2 content	mM./liter 24–33 (28) vol. % 53–75 (62)	– –	–	++	+	++	+	– –	–
$\frac{HCO_3^-}{H_2CO_3}$	20	–	N	–	N	+	N	+	N
pH	7.35–7.45 (7.4)	–	N	–	N	+	N	+	N
Urinary acidity and ammonia	<27 ml./kg. 0.1 N acid (+NH_3) per 24 hrs.	+	+	+	+	–	–	–	–

* Values in parentheses indicate means; + = increase; – = decrease; N = normal.

accumulate in the blood and tissues, the first two, being acid in reaction, combining with a portion of the fixed base supply and thus diminishing the alkali reserve of the body.

RENAL FAILURE. Acidosis is commonly observed in the terminal stages of nephritis and destructive renal lesions such as polycystic kidney, hydronephrosis, pyonephrosis, pyelonephritis, and renal tuberculosis. It is due in part to the fact that the impairment of renal functional efficiency eventually causes retention of those acid radicals which are normally eliminated in the urine, among the most important being phosphoric and sulfuric acids. Nephritic acidosis is also contributed to by the accumulation of certain organic acids of undetermined nature. As these acids accumulate in the blood and tissues they fix and render unavailable a portion of the base supply of the body, causing a diminution in and eventual depletion of the alkali reserve. Two other factors play an important part in contributing to the acidosis of advanced nephritis. The kidneys have lost their ability to conserve base (decreased Na-H exchange) (p. 330), which is lost in the urine and at times through the gastro-intestinal tract (vomiting). Furthermore, the ability of the kidney to form ammonia is diminished so that this mechanism, which in the presence of normal renal function aids in the conservation of the alkali reserve, fails to counteract the acidotic tendency associated with renal failure.

RENAL TUBULAR INSUFFICIENCY ("TUBULAR ACIDOSIS"). This is the term applied to a syndrome in which glomerular function is normal, but in which there is a defect in the ability to acidify the urine, accompanied by excessive excretion of K, PO_4 (p. 244), and Ca (p. 245). This disorder is apparently dependent upon a specific defect in the tubular mechanism of secretion of H ions, rather than to nonspecific tubular damage, and resembles the condition produced experimentally by inhibition of carbonic anhydrase activity in the renal tubular epithelium. Depression of carbonic acid (and, therefore, H^+) formation in the distal tubular cells results in excessive excretion of K into the lumen and passage of increased amounts of CO_2 into the blood stream (p. 331). The consequent increase in blood H_2CO_3 (i.e., H ions) exceeds the increase in urinary K excretion. There is therefore a net increase in urinary Na excretion, since a portion of the fraction of Na that is exchanged for H under normal circumstances now cannot be reabsorbed.

URETEROSIGMOIDOSTOMY. Transplantation of the ureters into the sigmoid (e.g., for bladder carcinoma) is often followed by the development of acidosis, with an increase in plasma Cl and a decrease in HCO_3. There is usually an increase in blood urea N, often without an increase in creatinine unless renal damage supervenes, e.g., as a result of pyelonephritis. These phenomena are probably due to reabsorption, from the bowel, of large amounts of urea, NaCl, and NH_4Cl (the ammonia of which is converted to urea).

ADMINISTRATION OF POTASSIUM. Administration of large amounts of potassium salts to subjects with normal kidney function results in the rather unique situation of decreased urinary acidity associated with increased acidity of the blood plasma. This has been explained on the basis that the increased concentration of K in the distal renal tubular cells depresses the Na-H exchange, K being excreted by these cells in increased amounts and the H ions usually exchanged for Na of the glomerular filtrate passing into the blood stream (p. 330).

Starvation. Starvation and carbohydrate restriction are associated with a state of acidosis due to ketosis. The underlying mechanism is identical with that occurring in diabetes mellitus, being dependent in the one case upon provision of insufficient quantities of glucose and in the other upon imperfect glucose utilization.

Anesthesia. The alkali reserve is diminished during anesthesia produced by ether and chloroform and, to a lesser degree, ethylene and nitrous oxide. It is believed by some that this may be due to the accumulation of excessive quantities of lactic acid as a result of anoxemia and imperfect glucose combustion. Ketosis and ketonuria frequently occur in the later stages of anesthesia and for some time after, but in all probability do not contribute in an important measure to the development of acidosis during the earlier period of anesthesia.

Dehydration (p. 311). The loss of excessive quantities of water from the body is invariably associated with the coincident or subsequent loss of a proportional amount of electrolytes, for the concentration of the latter in the body fluids must be maintained within rather narrow limits. Accordingly dehydration, if of extreme grade, is commonly associated with a state of acidosis due to the excessive loss of base from the body. This is particularly true of conditions in which the lost fluid contains relatively large quantities of base and is alkaline in reaction. For example, in diarrhea and in intestinal or pancreatic fistulae, large quantities of fluid, containing large amounts of base, are removed from the body with consequent dehydration and acidosis due to primary alkali deficit (p. 314). In dehydration due to excessive vomiting the matter is complicated by the fact that, the gastric contents being normally acid in reaction, large quantities of acid (HCl) are lost from the body, thus balancing the alkali deficit which might otherwise occur. As a matter of fact, alkalosis (alkali excess) is the most common sequel of excessive vomiting under ordinary conditions except in cases of achlorhydria or achylia. The inanition and carbohydrate restriction which are necessarily of common occurrence in conditions of continued vomiting may contribute to the production of alkali deficit because of the associated starvation ketosis. In a certain proportion of normal individuals there may be little or no free hydrochloric acid in the gastric juice, and continued vomiting, producing dehydration in such individuals, will eventually result in acidosis. Furthermore, in the vomiting of advanced nephritis (uremia) the gastric juice usually contains little or no free hydrochloric acid due to the fact that the organism seems to have lost its ability to conserve base, and under such conditions the acidosis of dehydration may aggravate that already existing as a result of advanced renal insufficiency. Acidosis is frequently an important feature of the continued recurrent or cyclic vomiting of children. The loss of hydrochloric acid from the stomach does not appear to counteract effectively the acidotic tendency in this condition, the alkali deficit being apparently due to the formation of excessive quantities of organic acids, particularly the ketone acids and lactic acid.

INGESTION OF ACIDS. Acidosis due to alkali deficit may be produced by the administration of excessive quantities of acids, e.g., hydrochloric, phosphoric, acetylsalicylic, which are used for therapeutic purposes. The same effect is produced by administration of ammonium chloride and ammonium nitrate, the ammonium being converted to urea and the anions (Cl, NO_3) displacing bicarbonate in the body fluids.

PREGNANCY AND TOXEMIAS OF PREGNANCY. The later stages of normal pregnancy are associated with a state of compensated acidosis due to slight alkali deficit, the cause of which has not been determined. In eclampsia, particularly during and shortly after convulsive seizures, the organism is in a state of uncompensated acidosis, pH values ranging from 7 to 7.2 having been observed in some instances. In the toxemias of early pregnancy, characterized by excessive and persistent vomiting, the acid-base balance is in a state similar to that existing in persistent vomiting due to other causes, acidosis being, however, much more frequently observed than alkalosis, as in the case of persistent vomiting in infants.

COMPENSATORY MECHANISMS. As the alkali reserve of the body diminishes certain compensatory mechanisms are set in operation in an attempt to maintain the hydrogen ion concentration within normal limits.

(1) *Increased Pulmonary Ventilation.* The respiratory center responds to an increase in hydrogen ion concentration due to primary alkali deficit just as it does to increased CO_2 tension, namely, by causing increased rate and depth of respiration. As a consequence of this increased pulmonary ventilation, CO_2 is washed out of the blood and the ratio of blood carbonic acid to blood bicarbonate tends to be restored to normal, the hydrogen ion concentration being consequently but little affected. During the early stages of alkali deficit, therefore, the organism is in a state of compensated acidosis, but as the condition progresses and the alkali deficit becomes more pronounced the compensatory mechanism fails and the condition becomes one of uncompensated acidosis with an increase in the hydrogen ion concentration of the blood.

(2) *Increased Ammonia Formation.* As the alkali reserve diminishes the organism attempts to conserve the base supply by increased ammonia formation in the kidney (p. 331). In nephritis, however, this mechanism fails to a certain degree, due to the fact that the ammonia-forming function of the kidney is impaired, perhaps proportionately to the impairment in its excretory function. Furthermore, as stated above, in the presence of renal failure the ability of the body to conserve base appears to be impaired, with the result that excessive quantities of base are eliminated in the urine.

(3) *Increased Acid Excretion.* In the majority of cases of acidosis due to primary alkali deficit increased quantities of acid are eliminated in the urine (increased Na–H exchange) (p. 331). This does not occur in the acidosis of advanced nephritis which is in itself partly due to failure of elimination of fixed acids by the kidneys. The increased elimination of electrolytes in the urine in this condition, and the diminution in the concentration of base and bicarbonate in the blood and tissue fluids, are invariably associated with the elimination of increased quantities

of water in an attempt to maintain the normal electrolyte concentration of the blood and tissue fluids. This diuresis results in dehydration, which is one of the constant features of this type of acidosis.

Biochemical Characteristics (Table 16). Acidosis of this type is commonly designated "metabolic acidosis." If uncompensated, it is characterized biochemically (plasma or blood) as follows: (1) disproportionate decrease in $[HCO_3^-]$; (2) decrease in $[H_2CO_3]$ (pCO_2); (3) decrease in CO_2 content ($[HCO_3^-]$ plus $[H_2CO_3]$); (4) decrease in $[HCO_3^-] : [H_2CO_3]$ ratio; (5) decrease in pH. If fully compensated, the CO_2 content is low, but the decrease in $[HCO_3^-]$ and $[H_2CO_3]$ is proportionate, the $[HCO_3^-] : [H_2CO_3]$ ratio and pH remaining within normal limits. The urinary ammonia and titratable acidity are increased (if kidneys are functioning normally).

Alkalosis

Alkalosis is a state in which either excessive amounts of acid are lost from the body without a comparable loss of base or alkali, or alkali is formed in or supplied to the body at a rate exceeding that of its neutralization or elimination. In terms of the bicarbonate system, alkalosis may result from either a primary decrease in the carbonic acid of the blood or a primary increase in blood bicarbonate (alkali reserve). As in the case of acidosis, a primary change in one of these factors is almost invariably associated with or followed by a secondary change in the same direction in the other factor, the hydrogen ion concentration of the blood under these circumstances being but little affected, the condition being one of compensated alkalosis. As the metabolic error progresses, however, the compensatory secondary change becomes insufficient to maintain the normal balance and the hydrogen ion concentration of the blood diminishes below the lower limit of normal (pH above 7.5), constituting a state of uncompensated alkalosis.

Primary H_2CO_3 Deficit (Respiratory Alkalosis). Excessive quantities of CO_2 may be washed out of the blood by hyperventilation of the pulmonary alveoli. This condition may be induced voluntarily by excessively rapid and deep respiration. Clinically, it is observed in the following conditions:

Hysteria. Alkalosis due to primary H_2CO_3 deficit is occasionally observed as a result of the hyperventilation which occurs at times during hysterical attacks.

Fever. Hyperventilation may occur as a result of the increased respiratory rate associated with an increase in body temperature. This is particularly prone to occur in inflammatory conditions involving the respiratory passages, especially pneumonia, in which condition the tendency toward H_2CO_3 deficit is balanced by a tendency toward retention of H_2CO_3 due to diminution in and functional impairment of the pulmonary ventilating surface.

High External Temperatures. Hyperventilation may be induced by exposure to high external temperatures such as hot baths. If prolonged, this exposure may result in alkalosis due to primary H_2CO_3 deficit.

Anoxic Anoxemia. Hyperpnea occurring in untrained individuals ascending to high altitudes where the atmospheric oxygen tension is low (anoxic anoxemia) commonly results in primary H_2CO_3 deficit and alkalosis.

ENCEPHALITIS. Alkalosis due to hyperventilation has been observed in some cases of encephalitis manifesting hyperpnea over prolonged periods of time.

DRUGS. Large amounts of sodium salicylate, such as are sometimes given in the treatment of acute rheumatic fever, produce stimulation of the respiratory center, with consequent hyperventilation and a tendency toward alkalosis of this type. Subsequent conversion to salicylic acid may result in a metabolic type of acidosis.

COMPENSATORY MECHANISMS. In view of the pathogenesis of this condition, the task of compensating for this defect falls on the kidneys. These organs, unless damaged, respond to the increasing alkalinity of the blood plasma by decreasing the Na–H exchange (p. 331) and ammonia formation (p. 331), thus returning less Na and HCO_3 to the blood plasma.

As the condition of alkalosis progresses, ketone bodies (diacetic and beta-hydroxybutyric acid and acetone) may accumulate in the blood due, presumably, to a disturbance of carbohydrate utilization in the liver. Ketonuria may occur under such circumstances. This ketosis, however, has but little effect upon the acid-base equilibrium.

BIOCHEMICAL CHARACTERISTICS (Table 16). Alkalosis of this type is commonly designated "respiratory alkalosis." If uncompensated, it is characterized biochemically (plasma or blood) as follows: (1) disproportionate decrease in $[H_2CO_3]$ (pCO_2); (2) decrease in $[HCO_3^-]$; (3) decrease in CO_2 content; (4) increase in $[HCO_3^-] : [H_2CO_3]$ ratio; (5) increase in pH. If fully compensated, the CO_2 content is low but the decrease in $[HCO_3^-]$ and $[H_2CO_3]$ are proportionate, the $[HCO_3^-] : [H_2CO_3]$ ratio and pH remaining within normal limits. The urinary ammonia and titratable acidity are decreased (if kidneys are functioning normally).

Primary Alkali Excess (Metabolic Alkalosis). Primary alkali excess or increase in the alkali reserve is the most frequent cause of clinically observed alkalosis. It occurs in the following conditions:

EXCESSIVE LOSS OF HCL FROM THE STOMACH. The loss of excessive quantities of hydrochloric acid from the stomach is encountered most frequently in individuals with pyloric or high intestinal obstruction and following protracted gastric lavage without proper provision for acid replacement. It is also at times observed in infants with pylorospasm and in patients with generalized peritonitis. As a result of the loss of the Cl ion from the blood there is present in the body an excess of base, chiefly sodium and potassium, which is retained in the form of bicarbonate. In this way a neutral salt (NaCl) is replaced by an alkaline salt ($NaHCO_3$). Alkalosis due to primary alkali excess is one of the most constant metabolic features of pyloric or upper intestinal obstruction, this disturbance of the acid-base balance, together with hypochloremia and nitrogen retention, constituting important diagnostic features of those conditions (see pp. 167, 289, 591).

ALKALI ADMINISTRATION. Alkalosis may follow the administration of excessive amounts of alkaline substances, particularly sodium bicarbonate, which is frequently given in large doses. The administration of sodium bicarbonate in the treatment of acidosis in chronic nephritis is particularly liable to result in alkalosis due to the difficulty of elimination of the excess alkali by the diseased kidneys.

POTASSIUM DEPLETION. Primary depletion of cell potassium results in a tendency toward alkalosis, with decrease in plasma Cl and increase in plasma HCO_3. This occurs clinically characteristically in Cushing's syndrome, and after administration of large amounts of ACTH or adrenocortical hormones (especially desoxycorticosterone) (p. 432). Under these circumstances, excessive amounts of K move out of the tissue cells (including renal tubular epithelium) into the extracellular fluids and therefore enter the glomerular filtrate in excess, escaping in the urine. In the presence of a decreased concentration of K in the distal tubular cells, excessive amounts of H ions (from carbonic acid formed in the cells) are excreted in exchange for Na, which, with the residual HCO_3 ions, passes into the blood stream in excessive amounts. The urinary excretion of the increased amount of K (originating in the intracellular fluid) must be balanced by corresponding amounts of anions, mainly Cl, drawn from the extracellular fluid compartment. This results in decrease in plasma Cl and an increase in plasma HCO_3, i.e., hypochloremic alkalosis.

Alkalosis of this type occurs in an apparently rare condition of non-infectious diarrhea in infants, in which there is probably a congenital defect in the mechanism of intestinal reabsorption of potassium. Large amounts of KCl are lost in the feces, with consequent depletion of tissue cell potassium and the sequence of metabolic aberrations outlined above. A similar situation develops in subjects with alkalosis, dehydration, and K depletion due to excessive vomiting, who have been rehydrated with fluids containing no potassium (i.e., NaCl, NH_4Cl, and glucose solutions). This results in additional loss of cellular K, which is replaced by Na (p. 282). As rehydration progresses, the concentration of K within the cells therefore falls, and the same sequence of events ensues. This prevents complete correction of the alkalotic state in such patients when only K-free solutions are administered.

ROENTGEN RAY AND ULTRAVIOLET IRRADIATION AND RADIUM THERAPY. A decrease in the hydrogen ion concentration of the blood plasma (increased pH) has been observed following deep x-ray therapy, radium therapy, and prolonged exposure to ultraviolet rays. The mechanism is not clear. In some instances (radiation sickness) it may be dependent upon excessive vomiting.

COMPENSATORY MECHANISMS. If functioning normally, the respiratory apparatus and the kidneys attempt to maintain the blood pH within normal limits. The increasing alkalinity of the blood plasma depresses the respiratory center (p. 354), with consequent slowing of respiration, hypoventilation, and decreased excretion of CO_2. The blood H_2CO_3 content increases accordingly.

The kidneys (unless damaged) attempt to rid the body of the excess HCO_3 by decreasing the extent of Na-H exchange (p. 331), thus returning less HCO_3 (more H_2CO_3) to the blood stream. Urinary acidity and ammonia are decreased; in severe cases the urine may become alkaline. Ketosis and ketonuria occur commonly in alkalosis due to excessive vomiting because of inadequate carbohydrate intake under such circumstances.

BIOCHEMICAL CHARACTERISTICS (Table 16). Alkalosis of this type is commonly designated "metabolic alkalosis." If uncompensated, it is characterized biochemically (plasma or blood) as follows: (1) disproportionate increase in [HCO_3^-]; (2) increase in [H_2CO_3] (pCO_2) : (3) increase in CO_2 content; (4) increase in [HCO_3^-] : [H_2CO_3] ratio; (5) increase in pH. If fully compensated, the CO_2 content is high, but the increase in [HCO_3^-] and [H_2CO_3] are proportionate, the [HCO_3^-] : [H_2CO_3] ratio and pH remaining within normal limits. The urinary ammonia and titratable acidity are decreased (if kidneys are functioning normally).

Alkalosis of this as well as the respiratory type is commonly accompanied by tetany, due perhaps to decreased ionization of calcium salts in the extracellular fluids (p. 240). The total serum calcium concentration remains within normal limits. Kidney damage, mainly degenerative changes in the tubules (nephrosis), occurs frequently, with oliguria and nitrogen retention. Another important feature, potassium depletion, at times manifested by decrease in serum K concentration, is discussed in detail elsewhere (p. 282).

METHODS OF STUDYING ACID-BASE BALANCE

The difficulty of investigating accurately the condition of a system which may be disturbed in one or more of so many ways must be apparent. Perhaps the most satisfactory method of approach, from a clinical standpoint, is to consider all disturbances of the acid-base balance in terms of the bicarbonate system, as has been done above, and to consider the hydrogen ion concentration of the blood as dependent upon the ratio between the concentrations of carbonic acid and bicarbonate in the blood. In other words,

$$cH = k\,\frac{H_2CO_3}{BHCO_3}$$

It must be remembered that a primary change in the concentration of either H_2CO_3 or $BHCO_3$ is associated with or followed by a secondary compensatory change in the same direction in the other factor, in an attempt to maintain the hydrogen ion concentration within normal limits. For example, if the $BHCO_3$ concentration (alkali reserve) is diminished, as in diabetes mellitus and nephritis, the development of uncompensated acidosis with an increase in the hydrogen ion concentration of the blood is for a time prevented by a compensatory decrease in the H_2CO_3 concentration of the blood (compensated acidosis). The subnormal carbonic acid content of the blood is in this instance a manifestation of acidosis. On the other hand, a primary decrease in the H_2CO_3 concentration is observed in conditions associated with hyperventilation, such as occurs at high altitudes, encephalitis, hysteria, etc., resulting in a tendency toward a decrease in the hydrogen ion concentration which is for a time balanced by a compensatory secondary diminution in blood bicarbonate (alkali reserve). In this case the decreased bicarbonate and carbonic acid concentrations of the blood are indicative of a state of alkalosis (compensated). It is evident that a clear-cut distinction must be made between primary and secondary changes in these factors, primary increase in carbonic acid and primary decrease in bicarbonate being indicative of a state of acidosis whereas secondary increase in carbonic acid and decrease in bicarbonate are indicative of a state of alkalosis. On the other hand, primary decrease in carbonic acid and primary increase in bicarbonate are indicative of a state of alkalosis whereas a secondary decrease in carbonic acid or increase in bicarbonate is indicative of a state of acidosis.

In considering the problem from this standpoint, three variable factors must be considered: (1) The hydrogen ion concentration (pH) of the blood, (2) the H_2CO_3 concentration (CO_2 tension) (pCO_2), and (3) the $BHCO_3$ concentration (alkali reserve). Obviously, a distinct disturbance of the acid-base balance may exist, as evidenced by a primary change in the concentration of either H_2CO_3 or $BHCO_3$, which, by virtue of a compensatory change in the other factor, is associated with no significant alteration in the hydrogen ion concentration. In other words, the primary disturbance is compensated. Under such conditions the true state of the acid-base balance can be determined accurately only by the determination of at least two of the three components of the equation cited above (hydrogen ion concentration, blood carbonic acid, and blood bicarbonate). Fortunately, however, in the great majority of clinical conditions associated with significant disturbances of the acid-base balance the fault lies primarily in a decrease or an increase in blood bicarbonate (alkali reserve), so that for most practical purposes investigation of this factor furnishes satisfactory although not exact information as to the state of the acid-base balance. Inasmuch as primary changes in either direction in either $[H_2CO_3]$ or $[BHCO_3]$ are followed by secondary changes (compensatory) in the same direction in the other factor, determination of the CO_2 content of the plasma (CO_2 present in both H_2CO_3 and $BHCO_3$) may be used as an index of the nature and magnitude of such changes. In fact, as indicated below, this determination is preferable to that of the CO_2 capacity (combining power). It must be realized that serious error may result from a failure to differentiate clearly between primary and secondary changes and that in doubtful cases too much reliance should not be placed upon the determination of any single factor.

CO_2 Content of Plasma. This is a measure of the amount of H_2CO_3 plus $BHCO_3$ present in the plasma of the subject, expressed either as millimols per liter (24–33, av. 28) or cc. CO_2 per 100 cc. plasma (volumes %) (53–75, av. 62). The determination is made in arterial blood (or blood taken from a vein after warming arm for 10–15 minutes), obtained under anaerobic conditions in order to avoid changes in pCO_2 that occur upon exposure of blood to air. With this procedure, both the H_2CO_3 and the $BHOC_3$ are maintained in the same condition in which they existed in the body.

As indicated in Table 16 (p. 336), the plasma CO_2 content is decreased in metabolic acidosis (p. 335) and respiratory alkalosis (p. 340), and is increased in metabolic alkalosis and respiratory acidosis. The complete quantitative picture can be obtained by determining, in addition, the pH of the blood plasma sample, which is a function of the ratio of $BHCO_3$ to H_2CO_3, as expressed in the Henderson-Hasselbalch equation. The ratio at any pH level may be calculated or be obtained from available tables or charts. Knowledge of the ratio and of the total amount of CO_2 present (H_2CO_3 plus $BHCO_3$) permits calculation of both H_2CO_3 and $BHCO_3$ with precision.

This procedure, i.e., determination of (*a*) CO_2 content and (*b*) pH, should be employed in all cases in which a high degree of accuracy is desired or in which the disease diagnosis is uncertain. In the latter case, there might be some question as to whether a low CO_2 content is a reflection of a metabolic type of acidosis or a respiratory type of alkalosis. Similar difficulty might be experienced in interpreting an increase in CO_2 content without the aid of the pH determination. However, in the great majority of cases, the nature of the underlying disease is known, e.g., diabetes mellitus, glomerulonephritis, pyloric obstruction, diarrhea, etc., and the direction of an associated disturbance in acid-base balance, i.e., acidosis or alkalosis, may be anticipated. Under these circumstances, clinical interest is centered in whether and to what degree the anticipated change is present. Determination of the CO_2 content alone may suffice for this purpose. Moreover, since clinical abnormalities in acid-base balance are usually of the metabolic type, decrease in CO_2 content signifies acidosis and increase alkalosis in the great majority of cases.

Carbon Dioxide Capacity of the Plasma (Table 16). The carbon dioxide capacity or CO_2 combining power of the blood plasma is expressed as the number of cubic centimeters of CO_2 which can be bound as bicarbonate by 100 cc. of blood plasma at 25° C. and 760 mm. Hg pressure at the normal pCO_2 of 40 mm. Hg. Inasmuch as the ability of the plasma to combine with CO_2 to form bicarbonate depends, in the final analysis, upon the quantity of available alkali present (alkali reserve), the determination of the CO_2 combining power or capacity of the blood plasma is a measure of the alkali reserve. The normal values for adults range from 53 to 78 cc. of CO_2 bound as bicarbonate by 100 cc. of blood plasma (53–78 volumes per cent) (24–35, av. 30 mM/liter). The normal values for infants are about 10 volumes per cent lower than those for adults. Decrease or increase in the CO_2 combining power is indicative of a corresponding change in the "alkali reserve."

Decrease in the CO_2 combining power, if but slight, is usually well compensated so that values ranging from 55 to 40 volumes per cent, indicative of a mild degree of acidosis, are generally associated with no significant alteration in the hydrogen ion concentration of the blood and consequently are not associated with clinical manifestations of acidosis. As the alkali deficit becomes more pronounced and the value for the CO_2 combining power drops to within the range of 40 to 30 volumes per cent, indicative of a state of moderate to severe acidosis, the compensatory mechanisms usually begin to fail, the acidosis becomes uncompensated, the hydrogen ion concentration is slightly increased and symptoms of acidosis are usually apparent. Values below 30 volumes per cent indicate a state of severe acidosis, practically always uncompensated, and therefore associated with a distinct increase in the hydrogen ion concentration. Values below 10 volumes per cent have been observed in individuals with eclampsia, severe diabetes, and advanced nephritis (uremia), figures below 15 volumes per cent being usually indicative of a rapidly fatal termination, except in diabetes.

Increase in the CO_2 combining power dependent upon primary alkali excess indicates a state of alkalosis. With values ranging from 80 to 90 volumes per cent the condition is usually well compensated, the hydrogen ion concentration of the blood being maintained within normal limits. However, since the great majority of the substances formed during metabolic activity in the tissues are acid in reaction, the compensatory mechanisms of the body which operate to maintain the normal acid-base balance are apparently much more efficient in dealing with a tendency toward acidosis than in preventing a change in the direction of alkalosis. Consequently, primary increase in the alkali reserve results in a state of uncompensated alkalosis more readily than uncompensated acidosis is effected by a change in the opposite direction. Therefore, as the CO_2 combining power rises above 90 volumes per cent, indicating a state of moderate to severe alkalosis, the condition rapidly becomes uncompensated and the hydrogen ion concentration the blood diminishes. In advanced alkalosis, such as is observed in some cases of pyloric and upper intestinal obstruction and in individuals receiving excessively large doses of alkali, the CO_2 combining power may reach 125 volumes per cent, although values above 110 are unusual.

Variation in the CO_2 combining power due to secondary compensatory changes in blood bicarbonate must be carefully distinguished from primary changes in this factor. A slight increase in the CO_2 combining power is commonly observed in acidosis associated with conditions in which there is a primary increase in blood carbonic acid, such as asphyxia, morphine narcosis, emphysema, etc. (respiratory acidosis). Similarly, a slight decrease in the CO_2 combining power may be observed in alkalosis dependent upon a primary decrease in blood carbonic acid, such as in hyperventilation of high altitudes, hysteria, encephalitis, etc. (respiratory alkalosis). These difficulties in interpretation can be resolved by determining, in addition, the plasma pH, as indicated above in discussing the plasma CO_2 content. This should be done in all doubtful cases. However, these conditions of primary H_2CO_3 excess and deficit are not commonly encountered clinically and are usually recognized without much difficulty. The great majority of clinical conditions associated with disturbance of the acid-base balance, such as advanced nephritis, diabetes mellitus, pyloric and acute intestinal obstruction, alkali overdosage, etc., fall under the headings of primary alkali deficit or primary alkali excess. In these conditions the determination of the CO_2 combining power of the blood plasma is useful in estimating the degree of acidosis or alkalosis in most instances. Although the values obtained by this method may be interpreted quantitatively in a manner satisfactory for most clinical purposes, they do not always indicate accurately the true quantitative variation in blood bicarbonate. This is due to the fact that the CO_2 capacity is determined after artificially saturating the separated plasma with CO_2 at its normal tension in arterial blood, i.e., 40 mm. Hg. If the condition is one of severe metabolic acidosis, the actual *in vivo* pCO_2 was much lower, and in metabolic alkalosis, higher. If there is significant deviation from the normal, therefore, plasma CO_2 combining power values do not reflect the true *in vivo* state of the H_2CO_3 : $BHCO_3$ balance with the same degree of quantitative accuracy as do values for CO_2 content, which is measured under conditions which preserve the *in vivo* relationships.

Alveolar CO_2 Tension. The determination of the H_2CO_3 content of the blood is of particular value in those conditions associated with primary H_2CO_3 excess or deficit. In certain of these conditions the determination of the CO_2 content of alveolar air may be employed to advantage. However, in pneumonia and emphysema, in which conditions primary H_2CO_3 excess in the blood, if present, is dependent upon imperfect diffusion through an altered respiratory membrane, the CO_2 content of the alveolar air cannot be assumed to be identical with that of arterial blood and hence indirect methods of determination of this factor should not be employed in these disorders. The chief clinical value of this method lies in the fact that the alveolar CO_2 content serves indirectly as an index of the concentration of blood bicarbonate (alkali reserve), since any increase or decrease in the latter factor, by causing depression or stimulation of the activity of the respiratory center with consequent hypoventilation or hyperventilation as the case may be, results in a compensatory increase or decrease in the concentration of carbonic acid in the blood and therefore in the CO_2 content of alveolar air.

The method of Marriott is perhaps most widely employed clinically. This procedure involves the collection and examination of rebreathed air, which differs from true alveolar air in that it has come into equilibrium with venous blood in the pulmonary capillaries whereas true alveolar air is in approximate equilibrium with arterial blood. The normal CO_2 values for men, obtained by the Marriott method, range from 5.2 to 5.7 volumes CO_2 per cent, corresponding to a CO_2 tension of 40 to 45 mm. Hg (pCO_2). The CO_2 tension of the alveolar air of women and children is from 3 to 5 mm. Hg lower than that of adult males. Due to the compensatory hyperventilation which accompanies the state of acidosis, these values are decreased in metabolic acidosis and, due to compensatory hypoventilation, are increased in metabolic alkalosis. Within a rather wide range of values, results obtained are in close agreement with those representing the CO_2 combining power of the blood. Mild acidosis is indicated by values ranging from 5 to 3.5 volumes CO_2 per cent (40–25 mm. Hg), moderate to severe acidosis by 3.5 to 2.5 volumes per cent (25–20 mm. Hg) and severe acidosis by values below 2.5 volumes per cent (below 20 mm. Hg). In diabetic coma, values as low as 8 to 10 mm. Hg have been observed. Figures above 6.5 volumes per cent (50 mm. Hg) are obtained in metabolic alkalosis. In alkalosis due to primary $CO_2(H_2CO_3)$ deficit in the blood, as is observed in the hyperventilation of high altitudes, hysteria, encephalitis, etc., the CO_2 tension and content of the blood and alveolar air are diminished, a fact which must be borne in mind in the interpretation of results obtained by this method in such conditions.

The Fridericia method of determination of alveolar CO_2 tension yields values which more accurately indicate the CO_2 (H_2CO_3) content of arterial blood, the figures being about 10 per cent lower than those obtained by the Marriott method.

Determination of the CO_2 content and tension of alveolar air has been generally replaced clinically by estimation of the CO_2 content or combining power of the plasma, which, being more direct indices of the state of the bicarbonate system of the blood, have to a large extent replaced the indirect procedure in routine practice.

Determination of pH of Blood Plasma or Serum. The hydrogen ion concentration of the blood plasma or serum may be determined by colorimetric or electrometric methods. The normal hydrogen ion concentration of blood plasma or serum ranges from pH 7.35 to 7.45, the average value being pH 7.4. The hydrogen ion concentration of the plasma of venous blood is very slightly greater than that of arterial blood, the pH of the former being about 0.03 lower than that of the latter. The clinical significance of hydrogen ion concentration determinations is limited because of the fact that abnormal values are obtained only during uncompensated stages of acidosis and alkalosis and therefore no information of positive value is afforded during the earlier, compensated stages, although during these periods evidence of a disturbance of the acid-base balance may be obtained by means of the estimation of the CO_2 combining power or content. The chief value of the determination of the hydrogen ion concentration of the blood in clinical conditions lies in the fact that when done in conjunction with one of the methods discussed above it indicates the degree of compensation or decompensation of the existing disturbance, and makes possible differentiation between metabolic acidosis and respiratory alkalosis as the cause of decrease in plasma CO_2, and between metabolic alkalosis and respiratory acidosis as the cause of increase in this factor (p. 345).

Values below pH 7.3 indicate a state of uncompensated acidosis, figures as low as 6.95 having been reported in advanced diabetes. Values below pH 7.0 are extremely rare, illustrating the fact that even in most severe acidotic states the blood practically always remains on the alkaline side of neutrality. Values above pH 7.5 indicate a state of uncompensated alkalosis, the highest values reported in clinical conditions being in the neighborhood of pH 7.6 although figures as high as pH 7.8 to pH 7.9 have been produced by experimental hyperventilation.

Other Methods of Investigation. ALKALI TOLERANCE (SELLARDS' TEST). The alkali tolerance test of Sellards depends in principle upon the fact that the administration of sodium bicarbonate to normal individuals in doses up to a maximum of 0.5 gm. per kilogram of body weight causes the urine to become alkaline in reaction. In the majority of normal individuals the ingestion of 5 to 10 gm. of sodium bicarbonate usually suffices to produce this effect. According to Van Slyke, in the presence of mild acidosis, 0.5 to 0.8 gm. may be required; in moderate to severe acidosis 0.8 to 1.1 gm. per kilogram of body weight are required; in severe acidosis more than 1.1 gm. per kilo are necessary to produce an alkaline reaction in the urine. In an individual of 60 kg. body weight, therefore, 0 to 30 gm. may be considered to be normal, 30 to 50 gm. may be assumed to indicate a state of mild acidosis, 50 to 65 gm. moderate to severe acidosis and over 65 gm. severe acidosis. The bicarbonate is administered in doses of 5 gm., dissolved in a little water, every two or three hours until the urine, which is voided before each dose, becomes neutral or faintly alkaline to litmus, the urine being thoroughly boiled before testing.

This test is of more negative than positive value. If the urine becomes alkaline following the administration of less than 0.5 gm. of sodium bicarbonate per kilogram of body weight it may be assumed that acidosis does not exist in that individual. However, higher values may be obtained in the absence of acidosis and the figures usually indicate a more severe grade of acidosis than actually exists. In other words, neither the presence nor degree of acidosis should be predicated on the basis of results obtained by this method. This is particularly true of the acidosis of advanced nephritis, in which condition the elimination of the ingested alkali is impaired because of the existing renal functional insufficiency. For this reason the administration of large doses of bicarbonate to individuals with renal failure is not without danger since the state of acidosis may be rapidly converted into one of advanced alkalosis.

DETERMINATION OF URINARY AMMONIA. A normal individual with an average diet eliminates approximately 0.7 gm. of ammonia in the urine daily, constituting 2.5 to 4.5 per cent of the total urinary nitrogen. As stated previously, the formation of ammonia in the kidney and its elimination in the urine in combination with acid radicals constitute one of the means whereby the body conserves its supply of available base. The necessity for elimination of increased quantities of acid, as in acidosis, is met by an increase in the quantity of ammonia formed by the kidney, the urinary ammonia being correspondingly increased. An increase in urinary ammonia, therefore, may be indicative of a state of acidosis, a decrease occurring in alkalosis. Values as high as 7 gm. of ammonia, comprising 50 per cent of the total urinary nitrogen, have been reported in diabetic acidosis.

Advanced grades of acidosis may be present in nephritis (uremia) with no comparable increase in the ammonia content of the urine. This is probably due to the fact that the ammonia-forming function of the kidney has been impaired to such an extent that this compensating mechanism fails to act as it does in the presence of normal renal function. Variations in urinary ammonia may be also produced by dietary factors, the ingestion of acid-forming foods causing an increase and base-forming foods a decrease in the daily output. In advanced hepatic disease urinary ammonia may be increased due to impairment of urea formation by the liver (see p. 169). Under such circumstances the urinary urea is correspondingly decreased.

TITRATABLE ACIDS IN URINE. The ability of the kidneys to excrete increased quantities of acid constitutes one of the mechanisms whereby the body compensates for any tendency toward an increase in the hydrogen ion concentration of the blood plasma. Since this mechanism is closely allied to that of ammonia formation and excretion, the determination of urinary ammonia plus the titratable acid of the urine gives some information as to the state of the acid-base balance. The following figures, expressed in terms of twenty-four hour excretion of N/10 acid plus NH_3, have been given by Van Slyke: normal resting adult, 0 to 27 cc. per kilo body weight; mild acidosis (compensated), 27 to 65 cc. per kilo body weight; moderate to severe acidosis, 65 to 100 cc. per kilo body weight, severe acidosis, over 100 cc. per kilo body weight. This determination is fairly reliable in indicating the presence or absence of acidosis in diabetes but cannot be utilized as a measure of the degree of acidosis in advanced cases.

Determination of Ketone Bodies. Ketosis, or the accumulation of ketone bodies (aceto-acetic acid, beta-hydroxybutyric acid, and acetone) in the body, is frequently, although not invariably, associated with acidosis. The concentration of ketone bodies in the blood of normal individuals ranges from 0.2 to 0.8 mg. per 100 cc., expressed as acetone. On a mixed diet small quantities of ketone bodies are eliminated in the urine of normal individuals, less than 125 mg. (expressed as beta-hydroxybutyric acid) being eliminated in twenty-four hours if sufficient quantities of carbohydrate are present in the diet. Factors which influence the rate of production, accumulation, and excretion of ketone bodies are discussed elsewhere (p. 94).

Ketosis and excessive ketonuria are commonly observed in starvation, during periods of carbohydrate privation, in normal pregnancy and in the toxemias of pregnancy, following ether anesthesia, in diabetes mellitus and at times in certain conditions associated with alkalosis, such as hyperventilation, intestinal obstruction and excessive alkali administration. In all of these conditions the fundamental cause of ketosis is probably the same (p. 94). Whether or not acidosis results from ketosis in any given case depends upon the quantity of ketone bodies produced, upon the condition of the alkali reserve and upon the other compensatory mechanisms whereby the body attempts to maintain the normal hydrogen ion concentration of the blood and tissue fluids, namely, hyperventilation, acid elimination in the urine, and ammonia formation by the kidney. Acidosis rarely attains a maximum degree of severity as a result of ketosis of normal pregnancy, starvation, or carbohydrate privation. It is in diabetes mellitus that the presence of ketosis is most significant, being practically always associated with true acidosis. The concentration of ketone bodies in the blood of patients with diabetic acidosis may reach values of 350 mg. or more per 100 cc. Large quantities of these substances may be eliminated in the urine, a twenty-four-hour output of 50 gm. or more being not infrequently observed. As stated above, ketonuria is frequently observed in alkalosis due to various causes. In that form dependent upon pyloric or upper intestinal obstruction with continued vomiting, starvation and carbohydrate privation play an important part in its pathogenesis. Moreover, alkalosis itself may increase ketogenesis by diminishing glucose utilization in the liver.

The acidosis of nephritis is not consistently associated with ketosis for obvious reasons. Clinically, the presence or absence of this condition is determined usually by the application of qualitative tests for the presence of acetone or diacetic acid in the urine (ketonuria). These qualitative tests serve as roughly quantitative procedures and are satisfactory for clinical purposes. Their chief value lies in the fact that ketonuria is one of the first clinical manifestations of beginning acidosis in diabetes and serves as a valuable therapeutic guide.

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CHAPTER XVI

Respiratory Exchange and Basal Metabolism

The maintenance of cell functions and of life is dependent upon the continuous supply of adequate amounts of oxygen to the tissues. In the course of metabolic activities of the cells, large quantities of CO_2 are produced, the bulk of which must be removed from the body. Both substances being gases, these exchanges of O_2 and CO_2 between the organism and the environment are accomplished by way of the lungs, comprising the beginning and end, respectively, of the process of respiration. Proper understanding of some of the fundamental aspects of these exchanges requires an understanding of certain of the so-called "gas laws."

1. At the same pressure and temperature, equal volumes of all gases contain the same number of mols.
2. In the absence of chemical reaction between gas and solvent, the amount of a gas which dissolves in a liquid is directly proportional to the pressure of the gas.
3. The pressure exerted by a gas at a given temperature depends upon the number of mols of gas in a given volume (molar concentration). This is referred to as the "partial pressure" or "tension" (p) of the gas, e.g., pO_2, pCO_2, pN_2.
4. The total pressure of a mixture of gases is equal to the sum of their partial pressures. Thus, the atmospheric (barometric) pressure (BP) is represented by $pO_2 + pCO_2 + pN_2$. This applies only to dry atmospheric air. The partial pressure of water vapor is a function only of temperature and is independent of the presence of other gases. Consequently, in calculating pO_2 and pCO_2, the following equation is applicable:

$$\text{BP} - pH_2O\text{ vapor} = pO_2 + pCO_2 + pN_2.$$

Inasmuch as the pressure exerted by a gas is determined by the number of mols of gas, and since the same number of mols of gases, under the same pressure, occupy equal volumes, it follows that the partial pressure of each component of a mixture of gases will be determined by the fraction which it occupies of the total volume of the mixture. In air, therefore,

$$pO_2 = \frac{(\text{BP} - pH_2O\text{ vapor}) \times \%\ O_2}{100}$$

$$pCO_2 = \frac{(\text{BP} - pH_2O\text{ vapor}) \times \%\ CO_2}{100}$$

$$pN_2 = \frac{(\text{BP} - pH_2O\text{ vapor}) \times \%\ N_2}{100}$$

In dry atmospheric air at a barometric pressure of 760 mm. Hg, the partial pressures of the constituent gases would be: $pO_2 = 20.9\%$ of 760, or 158 mm.; $pCO_2 = 0.04\%$ of 760, or 0.3 mm.; $pN_2 = 79\%$ of 760, or 600 mm. (Table 17).

The air in the pulmonary alveoli similarly contains O_2, CO_2 and N_2, but is also saturated with water vapor, which evaporates from the surface of the lining membranes at body temperature, exerting a partial pressure of about 48 mm. Hg. Inasmuch as the total gas pressure in alveolar air is the same as in the inspired (atmospheric) air, the partial pressure of each of the dry gases is exerted in a total of 760 minus 48 mm., or 712 mm. Hg. The same is true of the expired air. The percentage composition of, and pO_2 and pCO_2 in alveolar and expired air in a normal subject at rest are indicated in Table 17.

Table 17. Percentage Composition of, and pO_2 and pCO_2 in Inspired, Expired, and Alveolar Air in Normal Subject at Rest

	BAROMETRIC PRESSURE (mm. Hg)	H_2O VAPOR (mm. Hg)	OXYGEN CONTENT (vol. %)	pO_2 (mm. Hg)	CARBON DIOXIDE CONTENT (vol. %)	pCO_2 (mm. Hg)
Inspired air (dry)	760	0	20.9	158	0.04	0.3
Expired air	760	48	16.1	115	4.4	31
Alveolar air	760	48	14.2	101	5.6	40

In the lungs the blood gases come into approximate equilibrium with those of alveolar air. The separating membrane is very thin (1–2 μ), permitting ready diffusion of gases, and its surface area is very large (50–100 sq. m.). Blood passes through the lungs in about 0.75 second in a resting subject and in about 0.3 second during severe exercise, gas equilibrum being approximated more closely in the former state, which provides longer exposure of the blood to alveolar air. Under normal resting conditions, therefore, the pO_2 and pCO_2 of blood leaving the lungs (arterial blood) are about 100 mm. Hg and 40 mm. Hg, respectively.

In the tissue cells, where oxygen is being utilized and carbon dioxide produced, the pO_2 is relatively low (< 30 mm. Hg) and the pCO_2 high (50–70 mm. Hg).

Inasmuch as a gas flows from a higher to a lower pressure (diffusion gradient), O_2 passes from the arterial blood to the tissue cells and CO_2 passes in the opposite direction. Under ordinary conditions of blood flow in a normal resting subject, the pO_2 of the blood leaving the tissues (venous blood) is thereby lowered to about 40 mm. Hg and the pCO_2 increased to about 46 mm. Hg. The venous blood is then returned to the lungs, where it is arterialized, drawing O_2 from and losing CO_2 to the alveolar air, with which it comes into approximate equilibrium. These pressure relationships are illustrated in Figure 34.

At rest, a normal man absorbs about 250 cc. O_2 and eliminates about 200 cc. CO_2 per minute. During severe exercise, the volume exchange of these gases may increase more than ten-fold. The mechanisms of control of pulmonary ventilation

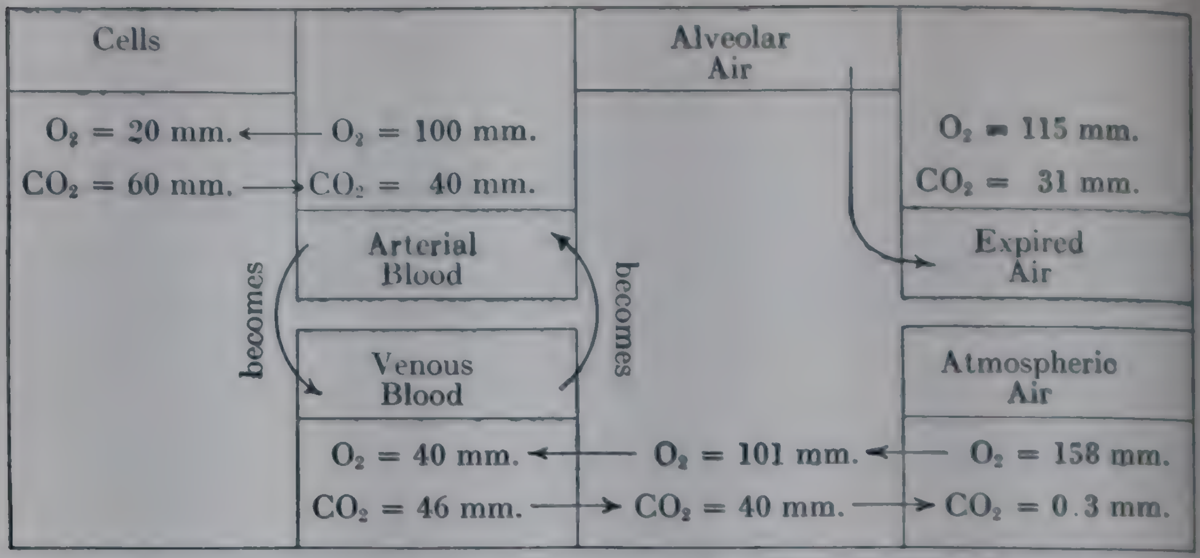

Fig. 34. Pressure relationships and direction of flow of oxygen and carbon dioxide between tissue cells, blood, lungs, and atmosphere.

are discussed in detail in texts on physiology, and the chemical aspects are summarized here only briefly.

CHEMICAL CONTROL OF RESPIRATION

The respiratory movements and pulmonary ventilation are controlled by nerve impulses arising in the respiratory center in the medulla. The activity of the latter is influenced, directly or indirectly, by changes in: (1) pCO_2, (2) pH, (3) pO_2, (4) blood flow, and (5) temperature. The indirect influences are mediated through chemoreceptors in the carotid and aortic bodies, which are stimulated chiefly by a decrease in pO_2 but also by an increase in pCO_2 and in H^+ ion concentration. Activity of the respiratory center is stimulated directly by an increase in pCO_2, and in H^+ ion concentration, the pCO_2 being the most important chemical factor in regulation of respiration.

Influence of CO_2 Tension and pH. Increase in the pCO_2 or acidity, either in the blood or locally in the respiratory center or aortic and carotid body chemoreceptors, results in stimulation of respiration and increased pulmonary ventilation. The reverse occurs with decrease in pCO_2 and H^+ ion concentration. The pCO_2 apparently exerts an influence in this connection beyond that which can be accounted for by the associated change in pH alone.

Under normal conditions, the extreme sensitivity of the respiratory center to minute changes in pCO_2 in the blood serves to maintain the latter within very narrow limits. The increased ventilation which accompanies any tendency toward a rise in pCO_2 (or H^+ ion concentration) results in prompt elimination of excess CO_2. Similarly, the slowed and shallower breathing, with diminished ventilation, which accompanies any tendency toward a drop in pCO_2 (or H^+ ion concentration) results in decreased excretion of CO_2. This constitutes an important part of the mechanism of acid-base regulation (p. 328).

Influence of O_2 Tension. Reduction in the pO_2 of arterial blood causes depression of the respiratory center, but stimulation of the carotid and aortic chemoreceptors. The latter effect exceeds the former, the predominant consequence being stimulation of respiration and increased ventilation. This is of importance in physiological adjustment to diminished atmospheric O_2 tensions during ascent to high altitudes. The stimulating effects of decrease in pO_2 are usually not manifest in the resting subject until it has fallen to rather low levels, but are more pronounced at higher levels during exercise.

TRANSPORT OF OXYGEN

The tissue cells of a normal man utilize about 250 cc. of oxygen per minute in the resting state and may consume over ten times that amount during strenuous exercise. At the pO_2 of alveolar air, a liter of blood is able to take up in physical solution only about 2 ml. O_2. Inasmuch as the maximum attainable blood flow is about 25 liters per minute, the maximum amount of O_2 that can be supplied to the tissue cells in this form is about 50 cc./minute, less than 2 per cent of the maximum requirement. Obviously, the oxygen must be transported in the blood chiefly in some form other than physical solution. This is accomplished by its combination with hemoglobin.

Table 18. Oxygen and Carbon Dioxide Contents and Pressures in Blood

	OXYGEN		CARBON DIOXIDE	
	CONTENT (ml./100 ml.)	pO_2 (mm. Hg)	CONTENT (ml./100 ml.)	pCO_2 (mm. Hg)
Arterial blood	17–22 (20)*	(100)	44–50 (48)	(40)
Venous blood	11–16 (13)	(40)	51–58 (55)	(46)
A-V difference	4–8 (6.5)		4–8 (6.5)	

* Mean values indicated in parenthesis.

Under conditions of complete saturation, the blood of a normal man (16 gm. Hb/100 cc.) holds a little more than 21 cc. of O_2, only about 0.3 cc. of which is in physical solution. Although of small magnitude, the latter factor is of considerable significance because, being a reflection of the pO_2, it comes into equilibrium in the lungs with the alveolar air O_2, and in the tissues with the interstitial fluid O_2. It is therefore one of the determinants of the amount of O_2 taken up and liberated by Hb, according to the equation,

$$O_2 + Hb \rightleftarrows HbO_2.$$

One mol of oxygen combines with 16,700 grams of Hb. Inasmuch as a mol of O_2 (at 0° C. and 760 mm. Hg) occupies 22,400 cc., 1 gram of Hb can combine with 22,400/16,700 or 1.34 ml. of O_2 (Hüfner factor). Consequently, the 16 grams of Hb in 100 cc. of blood, when fully saturated, can carry 21.4 cc. of O_2. However, under the conditions existing in the circulating blood, the Hb is not completely saturated with O_2, the amounts present in normal arterial and venous blood being indicated in Table 18. The degree of saturation depends upon the pO_2, this relationship being expressed in the dissociation curve of oxyhemoglobin (Fig. 35).

Dissociation of Oxyhemoglobin. The equation $O_2 + Hb \rightleftarrows HbO_2$ is shifted to the right or left as the pO_2 increases or decreases, with corresponding increasing saturation of Hb with oxygen (HbO_2) and increasing dissociation of HbO_2, respectively. However, the degree of saturation (or dissociation) is not directly proportional to the pO_2. If the blood is equilibrated with air containing O_2 at different partial pressures, and the quantity of O_2 (or HbO_2) in the blood is plotted against the pO_2, an S-shaped curve is obtained (Fig. 35). This is referred to as the dissociation curve of oxyhemoglobin and indicates the relative amounts of oxyhemoglobin (HbO_2) and reduced hemoglobin (Hb) present at different levels of oxygen tension.

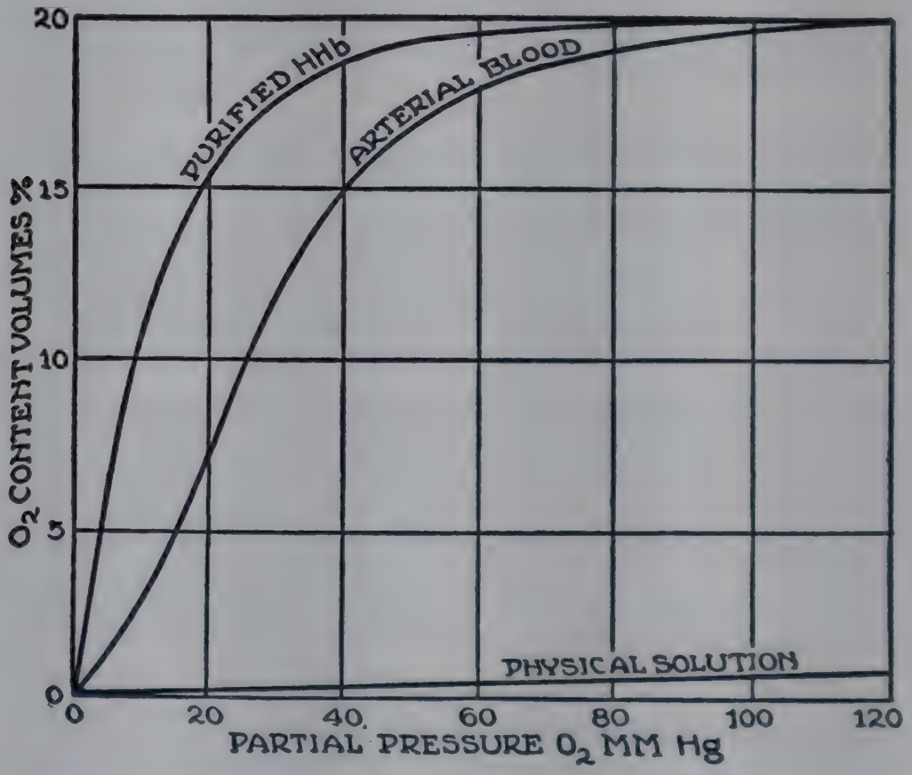

Fig. 35. Dissociation curve of oxyhemoglobin. The O_2 contents of arterial blood and plasma (physical solution) and a solution of purified HHb at various partial pressures of O_2. (After Barcroft, from Fulton: Howell's Textbook of Physiology, ed. 16, W. B. Saunders Co., 1950).

Certain features of this curve are of great physiological significance.

(*a*) Influence of pO_2. At a pO_2 of 100 mm. Hg., as in arterial blood (at pCO_2 40 mm. Hg and pH 7.4), Hb is 95 to 98 per cent saturated with O_2, i.e., 95 to 98 per cent is in the form of HbO_2 and 2 to 5 per cent in the reduced form. At a pO_2 of 70 mm. Hg, Hb is still 90 per cent saturated. The ability of the blood to carry oxygen therefore varies relatively slightly with variations in pO_2 above 70 mm. Hg. In fact, there is comparatively little reduction in Hb saturation, i.e., comparatively little dissociation of HbO_2, until the pO_2 falls below 50 mm. Hg, dissociation of HbO_2 increasing greatly, i.e., Hb saturation decreasing greatly, as the pO_2 falls below this level (Fig. 36; Table 19).

Because of this peculiar behavior of Hb in relation to the pO_2, adequate uptake of oxygen by the blood in the lungs is assured as long as the alveolar air pO_2 is above 80 mm. Hg. Adequate liberation of oxygen from the blood is assured in the tissues, where the pO_2 is usually below 30 mm. Hg.

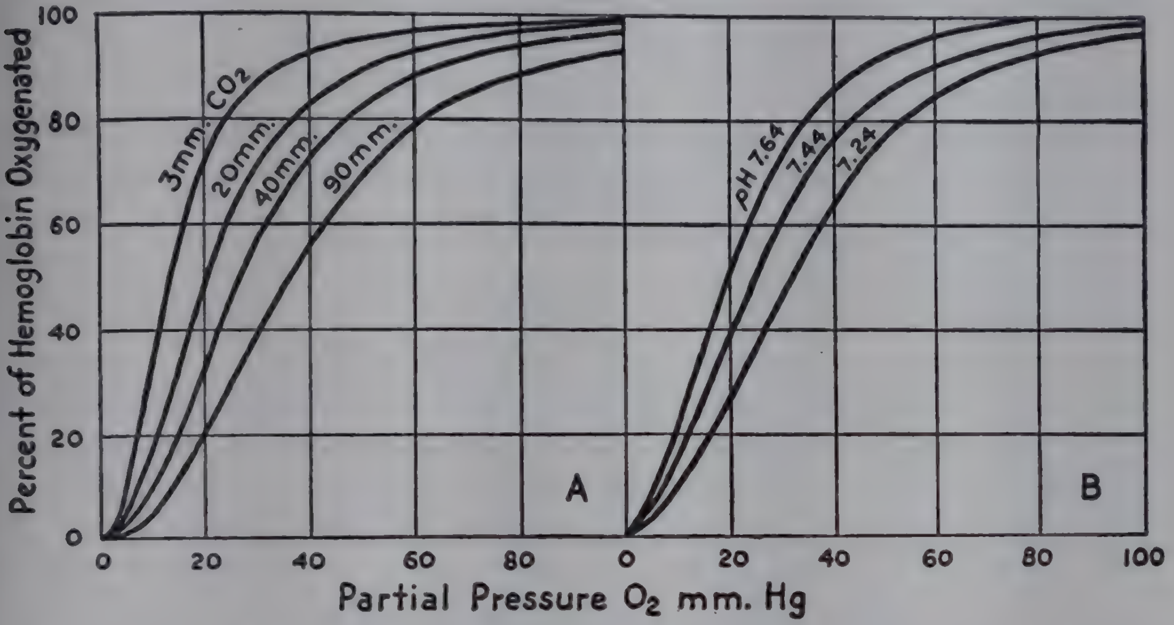

Fig. 36. The effects of increased pCO_2 and decreased pH upon the dissociation of oxyhemoglobin. Increased temperature also shifts the curve in the same direction as increased pCO_2. (After Peters and Van Slyke, from Fulton: Howell's Textbook of Physiology, ed. 16, W. B. Saunders Co., 1950.)

*Table 19. Effect of Oxygen and Carbon Dioxide Tensions on Oxygenation of Hemoglobin**

O_2 TENSION	PROPORTION OF HEMOGLOBIN COMBINING WITH OXYGEN AT FOLLOWING CO_2 TENSIONS			
mm.	CO_2 = 3 mm.	CO_2 = 20 mm.	CO_2 = 40 mm.	CO_2 = 80 mm.
	(%)	(%)	(%)	(%)
0	0	0	0	0
5	13.5	6.8	5.5	3.0
10	38.0	19.5	15.0	8.0
20	77.6	50.0	39.0	26.0
30	92.0	72.2	60.6	49.8
40	96.7	87.0	76.0	63.5
50	98.5	93.3	85.5	76.9
60	100	96.3	90.5	85.0
70	100	98.0	94.0	90.3
80	100	99.0	96.0	93.7
90	100	100	97.5	95.7
100	100	100	98.6	97.1

*(Henderson, Bock, Field, and Stoddard: J. Biol. Chem. *59*:379, 1924)

(*b*) INFLUENCE OF pCO_2 AND pH. With increasing CO_2 tension in the blood, the affinity of Hb for O_2 decreases (i.e., increasing dissociation of HbO_2, or decreasing saturation of Hb), the equation, $O_2 + Hb \rightleftarrows HbO_2$, being shifted to the left. This effect is more pronounced at relatively low than at high levels of pO_2, because of the shape of the dissociation curve (Fig. 36, Table 19). Thus, at 90 to 100 mm. Hg pO_2 (alveolar air), variations in pCO_2 from 20 to 80 mm. Hg. have comparatively little effect on HbO_2 dissociation (95–100% saturation of Hb). On the other hand, at a pO_2 of 20 mm. Hg (tissues), increasing the pCO_2 from 20 mm. to 80 mm. Hg causes the percentage saturation of Hb to fall from about 40 to less than 20.

Acids other than H_2CO_3, e.g., lactic acid, exert a similar effect, i.e., increase in the H^+ ion concentration of the blood increases dissociation of HbO_2. As in the case of the pCO_2, this influence is more pronounced at relatively low than at high pO_2 levels (Fig. 36).

Rise in temperature also increases dissociation of HbO_2, shifting the curve in the same direction as do increased pCO_2 and acidity.

As a result of these phenomena, adequate uptake of oxygen from the alveolar air is assured, despite the presence of CO_2, and its delivery to the tissue cells, facilitated primarily by the relatively low pO_2, is enhanced by the relatively high pCO_2, acidity, and temperature in the actively metabolizing tissues.

TRANSPORT OF CARBON DIOXIDE

In the resting state, about 200 cc. of CO_2 are produced per minute in the course of oxidative processes in the tissues, and are carried in the blood to the lungs, where they are excreted. During strenuous exercise, the quantity produced and transported increases enormously. The manner in which this relatively large amount of acid ($CO_2 + H_2O \rightleftarrows H_2CO_3$) is carried in the blood with only slight change in pH is discussed elsewhere (p. 326). Attention will be directed here chiefly to the various forms in which CO_2 exists in the blood and their quantitative distribution.

The direction of flow of CO_2 is determined by the pCO_2, which is 50–70 mm. Hg in the tissues, about 40 mm. in the arterial blood, rises to 46 mm. during passage of blood through the tissues (venous), and is 40 mm. in the alveolar air. The diffusion constant for CO_2 is much higher than for O_2 because of its greater solubility in body fluids; consequently it diffuses readily from the venous blood into the pulmonary alveoli despite the rather low pressure gradient (Fig. 34, p. 354).

CO_2 in Arterial Blood (Table 20). At rest, 100 cc. of arterial blood contains 45 to 55 cc. of CO_2 (volumes per cent), about 75 per cent of which is in the plasma and 24 per cent in the erythrocytes.

The 35.6 cc. (average) in the plasma are present in three forms: (1) physical solution (1.6 cc.); (2) HCO_3^- ions (34 cc.); (3) carbamino compounds of plasma proteins) (p. 359), in very small amounts (less than 0.7 cc.) because the plasma proteins contain relatively few NH_2 groups capable of combining with CO_2 under conditions existing in the blood. The 12.6 cc. (average) in the erythrocytes are present in the same forms: (1) physical solution (0.8 cc.); (2) HCO_3^- ions (9.6 cc.); carbamino compounds of Hb (2.2 cc.). About 90 per cent of the CO_2 in arterial blood is in the form of HCO_3^- ions (bicarbonate).

Entrance of CO_2 in Tissues (Table 20). At rest, 3.5 to 4.5 cc. (average 3.7) of CO_2 enter each 100 cc. of blood in the tissues, diffusing across the capillary walls in consequence of the tissue : blood CO_2 diffusion gradient. It enters the blood plasma in physical solution and diffuses readily from the plasma into the erythrocytes.

Table 20. Average Distribution of Carbon Dioxide in 100 ml. Normal Blood

CARBON DIOXIDE	ARTERIAL		VENOUS		DIFFERENCE	
	ml.	% of total	ml.	% of total	ml.	% of total
Total	48.2		51.9		3.7	
Total in plasma (60 ml.)	35.6	74	38.0	73	2.4	65
as dissolved CO_2	1.6	3	1.8	3	0.2	5
as HCO_3^-	34.0	71	36.2	70	2.2	60
Total in erythrocytes (40 ml.)	12.6	26	13.9	27	1.3	35
as dissolved CO_2	0.8	1.5	0.9	1	0.1	3
as carbamino-CO_2	2.2	4.5	3.1	6	0.9	24
as HCO_3^-	9.6	20	9.9	20	0.3	8

Carbon dioxide reacts with water as follows:

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$

In the plasma, this reaction proceeds very slowly, the equilibrium being far to the left. Consequently, the increment in CO_2 occurring in passing through the tissues drives the reaction to the right only slightly.

Within the erythrocytes, the reaction,

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-,$$

proceeds very rapidly, owing to the presence of carbonic anhydrase, an enzyme which catalyzes this reaction. Because of prompt removal of the ion end-products (H^+ and HCO_3^-), as indicated below, the fraction of the added CO_2 which diffuses into the erythrocytes is much larger than that which remains in the plasma (Table 20).

The relatively small capacity of plasma proteins for forming carbamino compounds is not influenced significantly by changes which occur in the blood during its passage through the tissues. Consequently, virtually none of the added CO_2 is transported in this manner (i.e., plasma carbamino CO_2 is unaltered). Within the erythrocytes, however, a considerable fraction of the added CO_2 is held in this form because of the fact that the capacity of reduced hemoglobin for forming carbamino compounds is more than three times that of oxyhemoglobin. Moreover, this capacity increases with increasing levels of pCO_2. In consequence, therefore, of the liberation of O_2 and addition of CO_2 in the tissues, about 20 to 25 per cent of the increment in blood CO_2 is transported by hemoglobin as carbamino-CO_2.

The largest fraction, by far, of the added CO_2 which enters the erythrocytes undergoes the reaction,

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-,$$

which proceeds rapidly because the end products, H^+ and HCO_3^- ions, are removed promptly. The added H^+ ions are very effectively "neutralized" by the enhanced buffering capacity of hemoglobin incident to the transformation of HbO_2 to Hb, reduced hemoglobin being a weaker acid than oxyhemoglobin (p. 326). The added HCO_3^- ions, being produced within the erythrocytes more rapidly than in the plasma, diffuse from the erythrocytes into the plasma, an equal number of Cl^- ions passing in the opposite direction (p. 327). Within the erythrocytes, the increase in negatively charged ions (HCO_3^- and Cl^-) is balanced by the K^+ ions "released" by the transformation of HbO_2 to Hb. In the plasma, the HCO_3^- ions entering from the erythrocytes are balanced by the Na^+ ions freed by the diffusion of Cl^- ions into the erythrocytes (Fig. 32, p. 327).

About 70 per cent of the added CO_2 is carried in the blood as bicarbonate (HCO_3^-), about 60 per cent in the plasma, the remainder in the erythrocytes. However, about 90 per cent of the increment in plasma HCO_3^- originates within the erythrocytes, as indicated above, largely as a direct consequence of the enhanced buffering capacity of the hemoglobin. Directly and indirectly, Hb is responsible for the transport of over 80 per cent of the CO_2 added to the blood in the tissues.

ANOXEMIA (HYPOXEMIA)

Anoxemia, meaning oxygen deficiency in the blood, and the more general term "anoxia (hypoxia)," meaning oxygen deficiency, should properly be applied to any condition of insufficiency of tissue oxidation processes. According to the factors involved, four types may be differentiated. These have been termed (1) anoxic anoxia, (2) anemic anoxia, (3) stagnant anoxia, and (4) histotoxic anoxia.

Anoxic Anoxia. This group includes conditions characterized by normal oxygen capacity but diminished oxygen tension in the arterial blood with a consequent varying degree of hemoglobin unsaturation.

(*a*) HIGH ALTITUDES. The condition commonly designated mountain sickness is in reality a state of anoxic anoxemia due to diminished oxygen tension in the atmospheric air and consequently in the alveolar air and blood stream.

(*b*) RAPID, SHALLOW RESPIRATION. Shallow breathing is conducive to inefficient oxygenation of the blood since, if the volume of tidal air is greatly decreased, comparatively little fresh air passes the physiologic dead space (150 cc.) to enter the alveoli. It is questionable whether this factor, in itself, is capable of producing anoxemia in disease states but it is unquestionably a contributory factor in such conditions as pneumonia, in which anoxemia is dependent largely upon other factors.

(*c*) MECHANICAL INTERFERENCE WITH OXYGEN ABSORPTION. This condition exists in pneumonia, pulmonary edema, pulmonary congestion, emphysema, bronchial asthma, acute bronchitis, and other diseases of the respiratory tract.

This type of anoxic anoxemia is the one most commonly observed clinically. Obviously, any condition which interferes with the passage of atmospheric air into the pulmonary alveoli or with the diffusion of oxygen from the alveoli into the blood will result in diminution in the oxygen content of the blood. It must be recognized, however, that a considerable portion of the ventilating surface of the lung may be functionally incapacitated with no alteration in oxygenation of the blood. Whether or not anoxemia occurs in these conditions is determined largely by (1) the circulation of blood in the affected area and (2) the rate of blood flow through the unaffected portions of the lung. In conditions such as unilateral pneumothorax and pleural effusion, the lung of the opposite side being normal, there is practically no circulation of blood in the collapsed lung. Since practically all of the blood returning to the heart in the pulmonary vein has passed through the normal lung and has consequently been adequately oxygenated, anoxemia does not occur. Similarly, in the early stages of lobar pneumonia, when the anatomic change consists of complete consolidation of a portion of the lung, anoxemia does not exist since little or no blood has circulated through the consolidated area. In bronchopneumonia, however, and in lobar pneumonia with an advancing lesion of a bronchial type, the circulation of blood through poorly aerated alveolar areas is relatively unimpaired and therefore the blood which returns to the heart in the pulmonary vein is partly oxygenated and partly nonoxygenated, cyanosis being a common manifestation under such circumstances. This anoxemic tendency is further aggravated by the frequently coexisting bronchitis and pulmonary edema. Furthermore, even in conditions such as pure lobar pneumonia, the rate of blood flow through the well aerated portions of the lung may be so increased that deficient oxygenation occurs as a result of the relatively brief exposure of the hemoglobin to alveolar oxygen. The degree of anoxemia has a distinct bearing upon prognosis in lobar pneumonia. Stadie found that in sixty-one fatal cases the degree of hemoglobin unsaturation varied from 14 to 52 per cent, the average being 32 per cent, a tremendous increase over the normal unsaturation value of 5 per cent. Fifteen of sixty patients who recovered at no time showed more than 16 per cent of arterial hemoglobin to be in the unsaturated form, a figure of 33 per cent being obtained at one time in one case.

(*d*) Congenital Heart Disease. In certain cases of congenital cardiac septal defects a portion of the blood may flow directly from the right to the left side of the heart without having passed through the lungs, the mixture of aerated and nonaerated blood in the systemic circulation resulting in a state of anoxic anoxemia. The condition is apparently further aggravated by incomplete oxygenation of the blood that does flow through the lungs. Anoxic anoxemia is relatively infrequently observed in congenital heart lesions associated with septal defects because the pathologic intracardiac deviation of blood flow is usually from the left to the right rather than from right to left.

The characteristic chemical feature of this type of anoxemia is an abnormally low oxygen saturation of the hemoglobin of arterial blood. Because of the compensatory polycythemia which usually occurs, particularly if the underlying condition is of a chronic nature, the oxygen content of arterial blood may be within normal limits or even actually increased. The oxygen content of venous blood may be normal or diminished depending upon the arterial blood values and upon the degree of oxygen utilization in the tissues.

Anemic Anoxia. This type of anoxemia is characterized by a diminution in the oxygen capacity of arterial blood due to a decrease in the amount of functioning hemoglobin.

(*a*) ANEMIA. The occurrence of anoxemia in anemia is readily understandable, the degree of oxygen saturation of arterial blood being normal but its oxygen content being diminished in proportion to the decrease in hemoglobin.

(*b*) CARBON MONOXIDE POISONING (p. 209). Carbon monoxide combines with the same group in the hemoglobin molecule as does oxygen, the combining capacity of hemoglobin for both gases being identical. However, the affinity of hemoglobin for carbon monoxide is more than 200 times as great as its affinity for oxygen, and therefore in the presence of relatively small concentrations of carbon monoxide in the air a considerable quantity is taken up by the blood with a consequent reduction in the amount of hemoglobin available for the transportation of oxygen to the tissues. Furthermore, the presence of carbon monoxide in the blood apparently diminishes the facility of dissociation of oxyhemoglobin and therefore increases the existing anoxemic tendency by interfering with the liberation of oxygen from the blood in the tissues.

(*c*) METHEMOGLOBINEMIA (p. 208). Methemoglobin is a substance, formed from reduced hemoglobin through the action of an oxidizing agent, which has the peculiar property of not being capable of combining with oxygen. Anoxemia associated with methemoglobinemia is of the anemic type inasmuch as it is due to a decrease in the quantity of functioning hemoglobin in the circulating blood.

(*d*) SULFHEMOGLOBINEMIA (p. 209).

Stagnant Anoxemia. Stagnant anoxemia is due to circulatory inefficiency, the rate of blood flow through the tissues being retarded with resulting increase in the percentage volume of oxygen removed from the blood in its passage through the capillaries. It is observed most commonly in circulatory failure associated with decompensated heart disease, in shock, and in conditions associated with vasospastic phenomena such as Raynaud's disease. In this type of anoxia the arterial oxygen tension, capacity and content may be normal, but because of the excessive oxygen loss in the tissues the venous oxygen tension and content are subnormal. In myocardial failure, anoxic anoxia is superimposed upon stagnant anoxia because of the impaired diffusion of oxygen from the alveolar air into the blood through the congested pulmonary alveoli.

Histotoxic Anoxia. Histotoxic anoxia is a term suggested by Peters and Van Slyke to indicate a condition in which the oxygen supply is normal in every respect but the degree of oxygen utilization by the tissues is diminished because the tissue cells are poisoned in such a manner that they cannot use oxygen properly. Histotoxic anoxia occurs in poisoning by alcohol, cyanide, and perhaps formaldehyde and acetone. In the absence of complicating factors such as shock, the arterial and venous oxygen tension, capacity and content are within normal limits.

ENERGY METABOLISM

Throughout the discussions of the metabolism of organic foodstuffs, attention has been focused particularly on the mechanisms and the nature of their chemical transformation. Various components of the tissues are undergoing degradation (catabolism) and resynthesis (anabolism) continually. Certain of the chemical reactions involved in these metabolic processes are exergonic, i.e., they are accompanied by liberation of energy, whereas others are endergonic, i.e., they require the introduction of energy.

Obviously, even under resting conditions, the maintenance of life and normal function requires the constant performance of work by a variety of tissues. This implies the utilization of energy, which must be supplied from external sources if the body stores of energy are to be preserved at a constant level. We shall concern ourselves here with the magnitude of the energy requirement under normal conditions and the factors that may influence it.

Caloric Value of Foods. Following the demonstration by early investigators in this field that production of heat in the body was dependent primarily on oxidative processes, attempts were made to relate the quantity of heat produced to the amounts of foodstuffs metabolized. This was feasible because the heat produced by burning (oxidizing) these substances outside the body can be determined readily in a combustion calorimeter, the average values (in Calories) obtained per gram being: glucose, 3.96; starch 4.2; animal fats, 9.5; animal proteins, 5.6 (vegetable proteins lower). A "Calorie" (kilocalorie) is the amount of heat required to raise the temperature of 1 kg. of water 1° C. (i.e., from 15° to 16° C.). In the body, protein does not undergo complete oxidation, a portion of its amino groups being converted to and excreted as ammonia and urea; this involves a loss of about 1.3 Cal. per gram, leaving 4.3 Cal. produced per gram of protein metabolized. Taking into consideration the variations in caloric value of individual carbohydrates, fats, and proteins, their average energy value when metabolized may be represented as follows (Calories per gram): fat, 9.3; carbohydrate, 4.1; protein, 4.1. When these corrected figures are applied, it is found that the amount of energy (Calories) produced by a given quantity of these foodstuffs in the body is the same as that (heat) produced by their combustion outside the body, within the limits of experimental error. Inasmuch as ingested foods are not completely assimilated, the caloric values of the fats, carbohydrates, and proteins of the diet are usually calculated as 9, 4, and 4 Cal./gram, respectively.

Heat Production. In living tissues, the bulk of the energy derived from the metabolism of organic compounds is liberated, not as a single burst of heat, as occurs in their combustion outside the body, but rather in a stepwise fashion through a series of integrated enzymatic reactions. Therefore, although in the complete oxidation of these compounds (e.g., glucose to CO_2 and H_2O) their full potential energy value is ultimately realized, relatively small fractions of the total are made available at each stage of their degradation. Presented in small parcels, energy is utilized much more efficiently than if it had been liberated explosively.

The energy thus produced is disposed of as follows: (1) a portion is primarily converted to heat for the maintenance of body temperature. (2) A portion is utilized for the performance of work, e.g., mechanical (muscle contraction), electrical (nerve impulse), secretory (glandular, intestinal, renal tubular cells, etc.), chemical (endergonic metabolic reactions). Such work is ultimately reflected largely in heat production. (3) A portion may be stored very temporarily in "energy-rich" phosphate bonds, or for longer periods in the form of substances (e.g., fat, glycogen) that may be called upon to provide energy at some future date. The over-all body composition and weight of normal adults does not vary appreciably from day to day; i.e., there is little or no accretion or loss of tissue, new components being formed only in amounts required for replacement (metabolic turnover, "dynamic state"). On an adequate diet, the organism is therefore in a state of equilibrium with its environment; this applies to its intake of energy as well as of H_2O, N, and inorganic elements. Consequently, an amount of heat must be lost, either actual (radiation, conduction, vaporization of water) or potential (constituents of urine and feces), that is equal to the caloric value of ingested proteins, fats and carbohydrates (i.e., caloric equilibrium). This has been demonstrated to be the case by direct measurement of heat loss (direct calorimetry).

Respiratory Quotient. In the process of oxidation, carbohydrates, fats, and proteins react with definite amounts of oxygen, forming definite amounts of carbon dioxide and water. The designation "respiratory quotient" or "R.Q." is applied to the ratio of the volume of CO_2 produced to that of O_2 utilized in this process (R.Q. = Vol. CO_2/Vol. O_2).

Table 21. Caloric, O_2, and CO_2 Equivalents of Carbohydrate, Fat, and Protein

	CARBOHYDRATE	FAT	PROTEIN
Calories per gram	3.7–4.3	9.5	4.3
Liters CO_2 per gram	0.75–0.83	1.43	0.78
Liters O_2 per gram	0.75–0.83	2.03	0.97
Respiratory quotient	1.0	0.707	0.801
Caloric value per liter O_2	5.0 Cal.	4.7 Cal.	4.5 Cal.

Carbohydrate R.Q. Complete oxidation of glucose may be represented as follows:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$
$$(1 \text{ mol}) + (6 \text{ mols}) \longrightarrow (6 \text{ mols}) + (6 \text{ mols})$$
$$(180 \text{ grams}) + (192 \text{ grams}) \longrightarrow (264 \text{ grams}) + (108 \text{ grams})$$

The glucose R.Q. is therefore 6/6, or 1.0. Inasmuch as 1 mol of gas occupies a volume of 22.4 liters, the volume of O_2 and CO_2 involved in this reaction is 22.4 × 6, or 134.4 liters. Consequently, 1 gram of glucose reacts with 134.4/180, or 0.75 liter of oxygen, forming 0.75 liter of CO_2. Since oxidation of 1 gram of glucose liberates 3.74 Cal., 1 liter of O_2 is the equivalent of 3.74/0.75, or 5.0

Cal. when glucose is oxidized. This (5.0 Cal.) is the caloric value of 1 liter of oxygen for glucose oxidation in the body.

FAT R.Q. Complete oxidation of tripalmitin may be represented as follows:

$$\begin{gathered} 2(C_{51}H_{98}O_6) + 145O_2 \rightarrow 102CO_2 + 98H_2O \\ (2 \text{ mols}) + (145 \text{ mols}) \rightarrow (102 \text{ mols}) + (98 \text{ mols}) \\ (1612 \text{ grams}) + (4640 \text{ grams}) \rightarrow (4488 \text{ grams}) + (1764 \text{ grams}) \end{gathered}$$

The R.Q. for tripalmitin is therefore 102/145, or 0.704. That for triolein is 0.712. The R.Q. for mixed fats in the body is regarded as 0.707. The volumes of O_2 and CO_2 involved in this reaction are 3248 liters (145 × 22.4) and 2284.8 liters (102 × 22.4), respectively. Consequently, 1 gram of tripalmitin reacts with 3248/1612, or 2.01 liters of O_2. Since oxidation of 1 gram of this fat liberates 9.5 Cal., 1 liter of O_2 is the equivalent of 9.5/2.01, or 4.7 Cal. when tripalmitin is oxidized. Essentially the same value applies to other animal fats and, therefore, 4.7 is the caloric value of 1 liter of oxygen for fat oxidation in the body.

Table 22. Average Elemental Composition and Metabolism of 100 Grams of Meat Protein (Loewy)

	100 GRAMS MEAT PROTEIN (grams)	EXCRETED IN FECES AND URINE (grams)	INTRAMOLECULAR WATER (grams)	REMAINING FOR OXIDATION (grams)
C	52.38	10.877		41.5
H	7.27	2.87	0.96	3.4
O	22.68	14.99	7.69	
N	16.65	16.65 (16.28 urine)		
S	1.02	1.02		

PROTEIN R.Q. Amino acids do not undergo complete oxidation in the body and, therefore, the type of equation written for carbohydrates and fats is not applicable to proteins. However, the approximate quantitative relationships between O_2 and CO_2 in the metabolic oxidation of amino acids may be calculated from data on the average composition and metabolism of meat protein. These are indicated in Table 22.

In its oxidation to CO_2, 12 grams of C combine with 32 grams of O_2; in forming H_2O, 2 grams of H combine with 16 grams of O_2. Consequently, 41.5 grams of C require 110.66 grams of O_2 and will produce 152.17 grams (3.46 mols) of CO_2, while 3.4 grams of H require 27.52 grams of O_2 [total O_2 utilized is 138.18 grams (4.32 mols)]. The R.Q. for protein oxidation is therefore 3.46/4.32, or 0.801. The volumes of O_2 and CO_2 involved were 96.7 liters (4.32 × 22.4) and 77.46 liters (3.46 × 22.4), respectively (per 100 grams protein). Since oxidation of 1 gram of protein liberates 4.3 Cal., 1 liter of O_2 is the equivalent of 4.3/0.967, or 4.5 Cal., which represents, therefore, the caloric value of 1 liter of O_2 for protein oxidation in the body.

As indicated in Table 22, the metabolism of 100 grams of protein gave rise to urinary excretion of 16.28 grams of nitrogen. Each gram of urinary N therefore represented the metabolism of: 100/16.28, or 6.15 grams of meat protein; 96.7/16.28, or 5.94 liters of O_2; 77.46/16.28, or 4.76 liters of CO_2. On the basis of analytical data for the average protein, it is generally estimated that 1 gram of urinary N represents: (1) the metabolism of 6.25 grams of protein; (2) utilization of 5.91 liters of O_2; (3) production of 4.76 liters of CO_2; (4) liberation of 26.51 Cal.

Example. The following data were obtained under basal conditions (overnight fast): (1) urinary N, 0.18 gram/hour; (2) O_2 consumption, 12.2 liters/hour; (3) CO_2 production 9.2 liters/hour. Information desired: (1) heat (Calories) production; (2) quantities of protein, carbohydrate, and fat metabolized; (3) percentages of total heat produced by oxidation of protein, carbohydrate, and fat, respectively.

0.18 gram of urinary N represents:
$0.18 \times 6.25 = 1.125$ grams of protein metabolized
$0.18 \times 5.91 = 1.06$ liters of oxygen
$0.18 \times 4.76 = 0.85$ liter of carbon dioxide
$0.18 \times 26.51 = 4.77$ Calories
Total O_2 (12.2 liters) minus protein O_2 (1.06 liters) = nonprotein O_2 (11.14 liters)
Total CO_2 (9.2 liters) minus protein CO_2 (0.85 liter) = nonprotein CO_2 (8.35 liters)
Nonprotein R.Q. = 8.35/11.14 = 0.75

As indicated in Table 23, a nonprotein R.Q. of 0.75 represents the liberation of 4.739 Cal. per liter O_2, 15.6 per cent of which comes from carbohydrate and 84.4 per cent from fat oxidation.

11.14 liters $O_2 \times 4.739 = 52.79$ Cal. (nonprotein)
15.6 per cent of 52.79 = 8.24 Cal. from carbohydrate (2.01 grams)
84.4 per cent of 52.79 = 44.55 Cal. from fat (4.79 grams)
52.79 + 4.77 (protein Cal.) = 57.56 total Cal./hour, of which
  1.125 grams protein provided 4.77/57.56, or 8.3 per cent,
  2.01 grams carbohydrate provided 8.53/57.56, or 14.3 per cent,
  4.79 grams fat provided 44.55/57.56, or 77.4 per cent.

SIGNIFICANCE OF R.Q. The chief practical value of the calculations discussed above lies in the fact that they permit estimation of the amounts of protein, fat, and carbohydrate metabolized during a given period, from knowledge of (1) the urinary N excretion, (2) the O_2 consumption, and (3) the CO_2 production.

If the organism (or isolated tissue, in vitro) were utilizing carbohydrate exclusively the R.Q. would be 1.0, if utilizing fats exclusively it would be 0.7, and if utilizing proteins exclusively it would be 0.8. Conversion of carbohydrate to fat results in R.Q. values greater than 1.0, because some of the oxygen of the carbohydrate, which contains relatively more of this element in proportion to carbon than do fats, becomes available for oxidative processes, decreasing the quantity of O_2 required from the inspired air. On the same basis, theoretically, conversion of fat to carbohydrate would produce R.Q. values below 0.7. Such values have been observed (e.g., in diabetes mellitus), but fatty acids have not been shown to produce a net increase in carbohydrate. The R.Q. for conversion of protein to glucose has been estimated as 0.632 to 0.706.

It has been found that in the average normal adult, studied under "basal" conditions, i.e., at complete mental and physical rest, fourteen to eighteen hours after taking food, the R.Q. is approximately 0.82. At this level, the caloric value of oxygen is 4.825 Cal./liter (Table 23). Progressive decrease in the proportion of carbohydrate being oxidized in the "metabolic mixture" (i.e., carbohydrate deprivation, diabetes mellitus) is reflected in progressive lowering of the R.Q., approaching 0.7 and occasionally falling below this value (excessive gluconeogenesis from protein).

Table 23. Respiratory Quotient and Caloric Equivalent of Oxygen for Different Mixtures of Fat and Carbohydrate

	PERCENTAGE OF TOTAL O_2 CONSUMED BY		PERCENTAGE OF HEAT PRODUCED BY		CALORIES PER LITER O_2
R.Q.	Carbohydrate	Fat	Carbohydrate	Fat	
0.707	0	100	0	100	4.686
0.75	14.7	85.3	15.6	84.4	4.739
0.80	31.7	68.3	33.4	66.6	4.801
0.82	38.6	61.4	40.3	59.7	4.825
0.85	48.8	51.2	50.7	49.3	4.862
0.90	65.9	34.2	67.5	32.5	4.924
0.95	82.9	17.1	84.0	16.0	4.985
1.00	100	0	100	0	5.047

CALORIMETRY

On the basis of what has been stated concerning heat production, it is evident that measurement of the amount of heat lost over any given period of time affords an approach to the estimation of the energy production (oxidative metabolism) of the body during that period. This may be accomplished either directly (direct calorimetry) or indirectly (indirect calorimetry).

Direct Calorimetry. The subject is placed in an insulated chamber (calorimeter) so constructed as to permit direct and precise measurement of heat loss from the body. Water is circulated through tubes within the chamber; the rate of flow is recorded automatically, as is the temperature of the water entering and leaving the chamber. Air is circulated through the calorimeter and measurements are made of the rate of flow, and of changes in temperature and water vapor. On the basis of these data, one may calculate the actual heat loss very accurately. Determinations may be made simultaneously of the consumption of oxygen and excretion of carbon dioxide by analyses of the air entering and leaving the chamber, permitting calculation of the respiratory quotient.

This procedure requires elaborate and expensive apparatus and is now chiefly of historical interest. It was found that calculation of heat production from data on O_2 consumption and CO_2 production gave results which approximated very closely those obtained by direct calorimetry. This observation formed the basis for the application of the widely employed procedure of indirect calorimetry.

Indirect Calorimetry. As illustrated by the example cited on page 366, the amount of heat lost during an experimental period can be calculated accurately from the following data: (1) urinary N excretion during that period; (2) O_2 consumption; (3) CO_2 excretion. The validity of this calculation rests upon the following facts: (1) 1 gram of urinary N represents the metabolism of 6.25 grams of protein, the consumption of 5.91 liters of O_2, the production of 4.76 liters of CO_2, and the liberation of 26.51 Cal.; (2) oxygen has a known fixed caloric value per liter at any given R.Q. level.

For usual clinical purposes, determination of urinary N may be dispensed with, inasmuch as 90 to 95 per cent of the heat produced is derived from oxidation of carbohydrate and fat. Two general types of apparatus may be employed: (1) the open-circuit system; (2) the closed-circuit system. The former is the more accurate; the latter is much more convenient, is sufficiently accurate for clinical purposes, and is used most widely.

Open-Circuit System. The subject breathes atmospheric air of determined composition, the expired air being collected in a rubber bag or a spirometer. Determination of the volume and O_2 and CO_2 contents of the expired air permits calculation of the volumes of O_2 absorbed and CO_2 produced and also, therefore, the R.Q. From the R.Q., one can ascertain the caloric value of 1 liter of oxygen, and, therefore, the heat production during the test period.

Closed-Circuit System. Several different types of apparatus are available, all of which are based on essentially the same principle. The subject breathes from and into a system filled with pure oxygen, the expired CO_2 and H_2O being trapped by soda lime. Decrease in the total volume of gas in the closed system is therefore due to and is a direct measure of oxygen consumption. The test period usually occupies six or eight minutes.

Inasmuch as CO_2 production is not measured, the R.Q. cannot be determined. However, the assumption that the R.Q. is approximately 0.82 (p. 367) under basal conditions is quite satisfactory for most clinical purposes. The caloric value per liter of O_2 is known to be 4.825 at this R.Q. Consequently, determination of the amount of O_2 consumed permits calculation of the approximate heat production during the test period.

BASAL METABOLISM

The terms "basal metabolism" or "basal metabolic rate" (BMR) are applied to the heat produced per unit time under "basal" conditions, i.e., at complete physical and mental rest, and in the postabsorptive state fourteen to eighteen hours after taking food. It was indicated previously that energy is derived by the organism from the metabolism (mainly oxidation) of exogenous (dietary) and endogenous proteins, carbohydrates, and lipids. This energy is dissipated in the form of heat and work, or is stored in the body. Under the specified "basal" conditions, the exogenous source of energy is removed and loss of energy in the form of work by voluntary muscles is virtually abolished. Under these conditions energy is expended in the maintenance of respiration, circulation, muscle tonus, gastrointestinal contractions, and body temperature, and the functional activities of various organs (e.g., kidneys, liver, endocrine glands, etc.). Although the respiratory, heart, and other involuntary muscles continue to function, their activity does not result in permanent increase in the potential or kinetic energy of the materials on which they act, viz., the blood, respiratory air, lungs, etc. Consequently, the organism, under these conditions, is deriving its energy solely from stored sources and is dissipating it almost exclusively in the form of heat. Inasmuch as the amount of energy lost must be equal to that produced, measurement of the heat loss over a given period of time is an index of the rate of energy production, i.e., metabolism. As indicated previously, this is usually done clinically by the indirect procedure, using a closed system.

Table 24. Calories per Square Meter per Hour (DuBois Standards) (Modified by Boothby and Sandiford)

AGE	MALES	FEMALES	AGE	MALES	FEMALES
5	(53.0)	(51.6)	20–24	41.0	36.9
6	52.7	50.7	25–29	40.3	36.6
7	52.0	49.3	30–34	39.8	36.2
8	51.2	48.1	35–39	39.2	35.8
9	50.4	46.9	40–44	38.3	35.3
10	49.5	45.8	45–49	37.8	35.0
11	48.6	44.6	50–54	37.2	34.5
12	47.8	43.4	55–59	36.6	34.1
13	47.1	42.0			
14	46.2	41.0	60–64	36.0	33.8
15	45.3	39.6	65–69	35.3	33.4
16	44.7	38.5			
17	43.7	37.4	70–74	34.8	32.8
18	42.9	37.3			
19	42.1	37.2	75–79	34.2	32.3

Physiological Variations in BMR. Basal metabolism varies with body size, age, and sex (Table 24). As would be anticipated, the magnitude of energy exchange increases with body size, but more directly in relation to surface than to either height or weight. Tables are available for derivation of surface area from the latter two factors. The BMR is therefore commonly expressed in one of three ways: (1) kilocalories/sq. meter body surface/hour; (2) liters of oxygen consumed/sq. meter/hour; (3) percentage above or below the mean normal for the subject in question. The last mode of expression is the one used most widely clinically, but it may occasionally obscure certain important facts, e.g., in obesity.

The average normal BMR for young adult men (20–30 years old) is about 40 Cal./sq. meter/hour (8.3 liters O_2/sq. meter/hour), increasing progressively below this age level (to 5 years) and diminishing progressively with increasing age. Normal values for females are about 6 to 10 per cent lower than for males (Table 24). For average individuals (e.g., 70-kg. man, 1.73 sq. meters), this would amount to 1300 to 1600 Cal. daily. As indicated above, deviations from the average normal may be expressed for clinical purposes in terms of percentage increase or decrease. Inasmuch as the BMR falls between ± 10 per cent of the mean in almost 90 per cent of normal subjects, plus 10 to minus 10 are usually regarded as acceptable normal limits, although certain normal subjects yield values beyond this range (to ± 15 per cent).

Table 25. Recommended Daily Caloric Requirements

	CALORIES PER DAY	
	MALE	FEMALE
Adults*		
Sedentary	2400	2000
Moderate activity	3000	2400
Strenuous activity	4500	3000
Pregnancy (latter half)		2400
Lactation		3000
Children		
16–20 years	3800	2400
13–15 years	3200	2600
10–12 years	2500	2500
7–9 years	2000	2000
4–6 years	1600	1600
1–3 years	1200	1200
Under 1 year	110/kg.	110/kg.

* Men 70 kg., women 56 kg.

Specific Dynamic Action (SDA) of Foods

Ingestion of food by subjects in an otherwise "basal" state results in an increase in heat production. This phenomenon is referred to as the specific dynamic action (SDA), or calorigenic action of foods. The extent of this increase above the basal level depends on the nature and quantity of the food. It is greatest for protein, less for carbohydrate, and least for fat. It is usually stated that ingestion of protein (25 gm.) equivalent to 100 Cal. gives rise to 130 Cal., and that ingestion of equicaloric amounts of carbohydrate and of fat gives rise to 106 and 104 Cal., respectively. The increments must be derived from body tissue sources and is "waste heat," not available for work.

The SDA of foodstuffs, therefore, is an expression of the "cost" of their metabolism, and adequate provision must be made to meet this expenditure if energy equilibrium is to be maintained. The values indicated above are of academic rather than practical importance, inasmuch as these foodstuffs administered together do not exert their SDA in an additive manner, the observed values being invariably lower than the calculated values. In calculating the dietary caloric requirement (mixed diet), it is recommended that the SDA be provided for as follows: 5 to 6 per cent of the total food calories should be added for a maintenance diet and 6 to 8 per cent for a liberal diet. More should be added (about 15 per cent) if the diet is very high in protein. On this basis, a subject with a BMR of 1600 Cal./day must be given an average mixed diet providing at least 1680 to 1696 Cal. in order to preserve caloric equilibrium.

The nature of the specific dynamic action of foods is not clear. It is not a reflection of the energy expended in digestive and absorptive processes, since it manifests itself after intravenous injection of amino acids. Amino acids vary in the magnitude of their SDA, which is apparently related to the metabolism of the nonnitrogenous moiety. For example, oxidative deamination, with transformation to urea, is accompanied by a larger heat increment than transamination. Phenylalanine, glycine, and alanine exert a particularly marked effect in this connection. There is evidence also that the increment is greater during the process of lipogenesis from glucose than during its oxidation to CO_2 and H_2O.

Total Metabolism (Caloric Requirement)

If a normal subject is to remain in energy (caloric) equilibrium, the daily diet must provide at least as much energy as is expended each day. The daily expenditure may be classified in the following categories: (1) basal metabolism (BMR), which is quite constant for any given individual, and is influenced, as indicated elsewhere (p. 369), by age (growth), sex, body size, environmental temperature (and barometric pressure), and such physiological states as pregnancy and lactation; (2) specific dynamic action of food; (3) muscular activity, the most important variable under normal conditions.

Table 26. Approximate Increments in Hourly Caloric Requirements (above Basal) for Different Occupations

OCCUPATION OR ACTIVITY	INCREASED REQUIREMENT CAL./HOUR
Sitting quietly	35
Reading aloud	40
Standing quietly	40
Tailor	70
Typing	75
Housework	110
Painter	145
Carpenter	150
Walking (moderate)	235
Sawing wood	380
Walking (fast)	550
Walking up stairs	1000

Extremely strenuous exercise may increase the energy expenditure more than ten-fold above the "basal" level (Table 26). In the case of a painter (1.7 sq. meters body surface) the daily caloric requirement might be calculated as follows:

8 hours sleep, at 65 Cal./hour = 520 Cal.
8 hours work, at 210 Cal./hour = 1680 Cal.
2 hours light exercise, at 170 Cal./hour = 340 Cal.
6 hours sitting quietly, at 100 Cal./hour = 600 Cal.
3140 Cal.
Specific dynamic action (6 per cent) = 188 Cal.
Daily caloric requirement = 3328 Cal.

In Table 26 are indicated the recommendations of the Food and Nutrition Board of the National Research Council with regard to the average caloric requirements at various levels of activity.

Clinical Significance of BMR

The value of the determination of basal metabolism in clinical practice lies largely in the exclusion of the thyroid gland as the cause of the patient's symptoms. A large volume of accumulated data indicates that about 75 per cent of individuals without thyroid disease have a basal metabolic rate ranging from plus 10 to minus 10 per cent, whereas 95 per cent of such individuals fall with the limits of plus 15 to minus 15 per cent (DuBois Standards). It is probable therefore, that the latter figures (plus 15 to minus 15) must be regarded as the range for all individuals not suffering with thyroid disease, although the narrower limits (plus 10 to minus 10) perhaps more accurately represent the strictly normal range. It must be emphasized, however, that hypermetabolism is by no means synonymous with hyperthyroidism, nor hypometabolism with hypothroidism (p. 373).

One high BMR reading does not prove the presence of thyrotoxicosis. The test is frequently performed under other than basal conditions. While fasting and physical rest can usually be enforced in a patient with average cooperation and intelligence, the third prerequisite for a "basal" condition, i.e., mental rest, is often beyond the control of physician and patient. Apprehension and the unpleasant sensations caused by the procedure may give rise to false high readings, to which the physician or technician may unwittingly contribute. If the patient is given to understand that a decision regarding operation may depend on the result of the test this will not make for mental relaxation. A poor night's rest, or worry of any sort, may be the cause of false high values. Results must be interpreted critically in the light of the entire clinical picture; it is frequently necessary to repeat the test several times and to get the patient accustomed to the procedure before a true BMR can be obtained. In case of doubt it is wise to perform the test under appropriate sedation, e.g., .06 gm. (1 gr.) of phenobarbital on the evening before the test and 0.03 gm. ($\frac{1}{2}$ gr.) one hour before the test in the morning.

In some instances it is possible to obtain a true BMR reading only under general anesthesia. It has been found that pentothal anesthesia lowers a normal BMR by up to 15 per cent. The high BMR resulting from thyrotoxicosis is practically uninfluenced by anesthesia, whereas the false high readings resulting from nervous factors are reduced to normal under anesthesia. This reduction of "false" high readings occurs immediately; the test is first performed on the unanesthetized patient and, with the mask still in place, sodium pentothal is administered intravenously. Care must then be taken, if necessary with the use of adhesive tape, to keep the mask in place and fitting snugly. Additional readings are then made with the patient completely relaxed under anesthesia.

Extremely high and extremely low external temperatures may cause a rise in the basal metabolic rate. Season, time of day, and sunlight are apparently without distinct effect. Some observers have reported lower values for Orientals than for Occidentals, but these findings have not been supported by recent observations. Certain drugs, particularly dinitrophenol and caffeine, to a lesser degree atropine, increase the metabolic rate, which is of course distinctly raised in normal individuals following the administration of thyroid extract, pituitrin, or epinephrine. The metabolic rate may fall slightly following administration of morphine.

Table 27. Conditions Associated with Low and High Basal Metabolic Rate

LOW BMR	ELEVATED BMR
Hypothyroidism	Hyperthyroidism
Panhypopituitarism	Acromegaly
Addison's disease	Pheochromocytoma
Nephrosis	Ingestion of thyroid hormone ("thyrotoxicosis factitia")
Starvation	Pregnancy, lactation
Anemia	Leukemia
Depressive psychosis or psychoneurosis	Polycythemia
"Hypometabolism" in normal individuals	Diabetes insipidus
	Fever
	Hypertension
	Technical errors: leak of apparatus, leak from patient (ill-fitting mouthpiece, absence of teeth, perforated eardrum)
	Nonbasal condition: nervous tension, apprehension
	Nonbasal condition: heart failure, dyspnea

In children, BMR determinations are of limited value, both because of difficulties in obtaining basal conditions, and because of uncertainties regarding normal standards.

In children under 6 to 8 years of age, BMR determinations cannot be performed satisfactorily with the usual (adult-type) apparatus and special equipment is necessary. The problem of obtaining basal conditions, not only physical rest and relaxation, but also absence of fear and apprehension, is considerably greater even in older children. They must be "trained" much more frequently than adults.

Another problem is that of adequate standards of reference, especially in children of abnormal body build. Computations on the basis of body surface, of height, and of body weight show considerable disagreement. Hypothyroid children show low values, calculated either on the basis of surface or of height. Obese children often show low values when calculated on the basis of surface or weight, but not when height tables are used.

Increased Basal Metabolic Rate

Hyperthyroidism. Increase in the basal metabolic rate is perhaps the most characteristic manifestation of hyperthyroidism, the degree of rise paralleling the severity of the condition. Hyperthyroidism may occur without any apparent gross anatomic change in the thyroid gland, or in association with exophthalmic goiter, adenoma of the thyroid or, less commonly, malignancy or acute inflammatory processes in the thyroid gland.

The action of thyroxine or thyroid extract in the body is prolonged; following their administration, metabolism rises to a maximum in from six to eight days and then gradually declines, reaching the resting level after a variable period of time depending upon the amount of the substance administered. The effects of a single injection of thyroxine may persist for 30 to 60 days. In subjects with myxedema, 1 mg. of thyroxine causes an increase of about 2.8 per cent in basal metabolism. Normal subjects are much more resistant to thyroid hormone, responding relatively slightly to doses that produce a pronounced effect in the presence of hypothyroidism.

The basal metabolic rate is usually a satisfactory index of the severity of the disease (hyperthyroidism) and is invaluable for determining the operative risk in any case. Values above plus 100 per cent have been observed, cases showings values of plus 20 to plus 50 being considered as mild or moderately severe, plus 50 to plus 75 severe, and above plus 75 very severe. Thus, determination of the basal metabolic rate is valuable from the standpoints of diagnosis, prognosis and the determination of operability in any given case.

Other Endocrine Disorders. Elevated values (plus 20 to plus 35) are occasionally found during relatively brief periods in the early stages of acromegaly. This increase in the basal metabolic rate is due to hypersecretion of the pituitary gland (eosinophilic adenoma, p. 397), a condition which exists for a period in the life history of acromegaly, being later often superseded by normal secretion or hypopituitarism with consequent diminution in the basal metabolic rate. In the great majority of cases the reported values are well within normal limits. Increased values may also be found in patients with Cushing's syndrome (basophilic adenoma).

Administration of epinephrine is rapidly followed by an increase in the metabolic rate. Clinical states of hyperadrenalism are observed during certain stages of the growth of tumors of the adrenal gland, such as pheochromocytomas, and may be associated with an increase in the basal metabolic rate. It is believed by some that this action of epinephrine is due not to a specific calorigenic effect as in the case of thyroxine, but rather to the generalized increase in muscle tonus which results from sympathetic stimulation.

Pregnancy. During normal pregnancy the BMR rises gradually until, in the last trimester, it reaches values of about 20 to 25 per cent above the prepregnancy level. This rise probably does not signify a change in metabolism or in thyroid activity of the maternal organism but rather is due to the increase in the bulk of active tissue; in other words, the values obtained represent the sum of oxidation processes of the maternal and fetal tissues. High levels of protein-bound iodine have been found in the blood of pregnant women. Inasmuch as there are no clinical manifestations of hyperthyroidism in normal pregnant women, the significance of this finding is not clear.

Fever. An increase in the basal metabolic rate occurs in practically all fevers, the average rise being about 13 per cent for each degree Centigrade or 7.2 per cent for each degree Fahrenheit. A relatively greater rise occurs in cases in which toxemia is increased out of proportion to the fever, the destruction of body protein in such instances being increased. In tuberculosis, on the other hand, the rise in the basal metabolic rate is relatively less than in other febrile disorders, due to the fact that in that condition the toxemia is usually comparatively slight and there is relatively little toxic destruction of body protein. The malnutrition which is commonly associated with tuberculosis may also be a factor in the production of this tendency toward a relatively low metabolic rate.

Diseases of the Blood. The basal metabolism is increased in the great majority of patients with both lymphatic and myelogenous leukemia even in the absence of fever, the rise being in many instances comparable to that observed in hyperthyroidism. The exact cause of this phenomenon is not known; the basal metabolic rate does not appear to parallel the total leukocyte count but may be dependent rather upon the increased activity of the bone marrow or lymphatic apparatus which may not be accurately indicated by the number of immature cells in the circulating blood. An increased rate of cell turn-over may be an important factor, as indicated by the high blood uric acid concentration in many cases, particularly in chronic myelogenous leukemia.

High values ranging from 16 to 40 per cent may be observed in true polycythemia or erythremia, although in some cases the basal metabolic rate may be within normal limits. It has been suggested that the increase may be due to the excessive destruction of nuclear material as evidenced by increased uric acid production.

Anemia, particularly pernicious anemia and some cases of splenic anemia, may be associated with an increased metabolic rate, the cause of which is unknown. It is probably due to the underlying condition rather than to the anemia itself. In some cases of severe secondary anemia the basal metabolic rate may be increased.

Essential Hypertension. In the great majority of cases of essential hypertension the basal metabolic rate is within the normal limits of plus 15 to minus 15 per cent. However, in some instances values as high as plus 70 per cent may be observed, figures above plus 20 per cent having been obtained in 3.4 per cent of a large series of cases. The cause of the increased metabolic rate in these cases in the absence of myocardial insufficiency and dyspnea is not clear. Some observers have attributed it to increased thyroid secretion and others to increased epinephrine secretion.

Myocardial Insufficiency. Heart disease in itself has apparently no effect upon the metabolic rate. However, values as high as plus 40 per cent may be obtained in patients with myocardial failure. This is due partly to the increased activity of the respiratory muscles incident to the dyspnea associated with heart failure and partly to the increased oxygen consumption which results from slowing of the circulation in the tissues.

Diabetes Insipidus. Diabetes insipidus may be associated with an increase in the basal metabolic rate amounting in some instances to as much as 30 to 45 per cent. The explanation for this phenomenon is not clear. Diminution in the urine output following the administration of pituitrin is followed by a return of the basal metabolic rate to a normal level.

Decreased Basal Metabolic Rate

Hypothyroidism. Cretinism, myxedema and minor grades of hypothyroidism are associated with a decreased basal metabolic rate, the extent of the decrease being an accurate indication of the degree of thyroid functional insufficiency. Values below minus 35 per cent are uncommon although figures as low as minus 42 per cent have been observed in severe cases of myxedema. The statement made with regard to the value of the determination of the basal metabolic rate in hyperthyroidism apply equally to hypothyroidism, its determination being almost essential for the diagnosis of mild grades of hypothyroidism and in following the response to therapy in severe cases.

Other Endocrine Disorders. Hypoadrenalism, either functional or associated with organic disease of the adrenal glands such as Addison's disease, may be associated with a decrease in the basal metabolic rate, usually not, however, to the degree observed in hypothyroidism. Values below minus 25 per cent are extremely unusual. Similar findings may be obtained in hypopituitarism, which may occur either as a congenital disturbance or as a manifestation of the destructive action of tumors of the pituitary gland. Very low figures, at times below minus 40 per cent, are characteristically obtained in patients with pituitary cachexia (Simmonds' disease). This condition is characterized primarily by marked or complete atrophy of the hypophysis with extreme hypofunction and at times atrophy of the entire endocrine system.

The Nephrotic Syndrome. The basal metabolic rate is usually subnormal in patients with "lipoid nephrosis," being frequently about minus 20 per cent and occasionally as low as minus 35 per cent. These low values are most commonly obtained during the periods in which edema is marked but cannot be explained satisfactorily solely upon the basis of the edema in many cases.

Shock. The basal metabolism may be strikingly diminished in shock, the degree of diminution being of some value from the standpoint of prognosis.

Malnutrition. The basal metabolic rate may be low in conditions associated with malnutrition or starvation. Protein privation appears to be the most important factor in these cases. Normal individuals receiving a low caloric diet deficient in protein may exhibit basal metabolic rates as low as minus 25 to minus 30 per cent. Similarly, individuals with diabetes mellitus and nephritis, who are frequently malnourished, may show similar findings. Subnormal values, at times as low as minus 40 per cent, have been reported in patients with anorexia nervosa, a condition which may be difficult to distinguish from pituitary cachexia (Simmonds' disease). The low basal metabolic rate in anorexia nervosa is probably due largely to inanition.

Miscellaneous. Subnormal metabolic rates may be obtained at times in patients with severe anemia, chronic arthritis, peptic ulcer, and a variety of diseases of the nervous system, including psychoneurosis, schizophrenia, and autonomic imbalance (vagotonia).

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CHAPTER XVII

Hormone Assay and Endocrine Function

(WITH A. E. RAKOFF)

The functional state of some of the glands of internal secretion can be determined at the present time only by indirect methods, i.e., by studying certain phases of metabolism which are influenced specifically by certain hormones. For example, the state of parathyroid function is most satisfactorily ascertained clinically by studies of calcium and phosphorus metabolism and serum phosphatase activity. Abnormal function of the pancreatic islet cells is reflected in abnormalities of carbohydrate metabolism, and thyroid function in the basal metabolic rate, cholesterol and carbohydrate metabolism, and creatine metabolism. Water metabolism is affected by the antidiuretic hormone of the posterior lobe of the hypophysis and carbohydrate metabolism by epinephrine. Carbohydrate, lipid, and protein metabolism are influenced by adrenal cortical and growth hormones and the adrenotrophic hormone of the anterior hypophysis, and protein metabolism also by the male sex hormone. The adrenal cortex exerts a specific effect upon the metabolism of sodium, potassium, chloride, and water. The manner in which these metabolic abnormalities may be used as an index of the state of function of various endocrine glands has been indicated elsewhere, in discussing the metabolism of each of the substances in question.

A more direct approach is possible in the case of certain hormones, quantitative determination of their concentration in the blood or urine affording a more or less exact index of the functional activity of the organs in which they originate or of those concerned with their intermediary metabolism. Thus, the concentration of organic iodine in the blood plasma or serum is a measure of the quantity of circulating thyroid hormone (p. 270). Assays can also be made of certain hormones of the anterior hypophysis, the ovary, testis and placenta and the adrenal cortex. Because of the importance of this field of clinical investigation, and because it is fundamentally biochemical in nature, the clinical significance of data obtained by such procedures will be discussed, even though the methods employed in certain instances are biological rather than chemical.

PITUITARY HORMONES

Three anatomical divisions of the hypophysis yield extracts which, on injection, exert well-defined effects in various animal species. These divisions are (1) the neurohypophysis (posterior lobe), (2) the pars intermedia, and (3) the anterior lobe (adenohypophysis). In man and other mammals, the latter is by far the most important, inasmuch as it controls the functional activity and structural integrity of other important endocrine glands, e.g., the thyroid, adrenal cortex, and gonads, through its so-called "trophic" hormones. In addition, it produces factors which exert direct actions in certain fundamental phases of intermediary metabolism, viz., the growth hormone.

ANTERIOR PITUITARY HORMONES

Following hypophysectomy, the thyroid gland, adrenal cortex, and gonads undergo atrophy, and the animal loses weight. In addition in young animals, longitudinal growth is arrested, closure of epiphyseal lines is delayed, and the gonads fail to mature, the animals remaining infantile. Certain metabolic defects are apparent. Oxidative processes are depressed; liver and muscle glycogen are depleted; fat oxidation is decreased; ketosis does not appear following procedures which usually induce increased ketogenesis; fat turnover is decreased, as reflected in its decreased mobilization from the fat depots. These animals are exceedingly sensitive to insulin.

All of these morphological and metabolic abnormalities can be prevented or corrected by administration of anterior pituitary extracts. In recent years, several anterior pituitary principles have been separated and purified, which exert rather characteristic effects and which are generally assumed to be different hormones. All are protein in nature. They may be placed in two categories. (A) The so-called "trophic" hormones are responsible for the structural integrity and functional activity of certain specific "target" endocrine glands. Their effects are due to stimulation of their respective target glands, and they exert no effect in the absence of these organs. They include the following: (*a*) thyrotrophic, (*b*) adrenocorticotrophic and (*c*) gonadotrophic hormones ((1) follicle-stimulating, (2) luteinizing, and (3) lactogenic). (B) At least one factor, the growth hormone, exerts direct metabolic effects on protein, carbohydrate, and fat metabolism, not mediated by other endocrine glands.

Regulation of Anterior Pituitary Function. The anterior pituitary has been referred to as the "master gland" because, through the trophic hormones, it regulates the activities of a large portion of the endocrine system. Its own secretory activities are controlled in part by "back action" of circulating hormones of dependent glands (gonads, thyroid, adrenal cortex), and in part by cerebral influences, apparently mediated by a humoral mechanism.

BALANCE BETWEEN TROPHIC HORMONES AND SECONDARY HORMONES. Each of the trophic hormones of the pituitary stimulates a dependent gland to secrete its hormone. The latter, in turn, has an inhibitory influence on the secretion of the respective trophic hormone; it "puts the brake" on the pituitary. Consequently, each pair, i.e., gonadotrophic hormone and gonadal hormones, thyrotrophic hormone and thyroid hormone, adrenocorticotrophic hormone and adrenocortical hormones, represents, under physiologic conditions, a balanced unit. Removal of the secondary hormone-producing gland (gonadectomy, thyroidectomy, adrenalectomy) releases the respective pituitary trophic hormone secretion, and by "removing the brake," leads to excessive secretion of gonadotrophic, thyrotrophic and adrenocorticotrophic hormone, respectively. Excess production of the dependent hormone suppresses pituitary secretion of the respective trophic hormone. The latter can be suppressed experimentally by administering exogenous gonadal, thyroid, or adrenocortical hormones.

If excessive amounts of one of the secondary hormones, produced endogenously or administered experimentally or therapeutically, act over longer periods of time, they will suppress not only the specific trophic hormone but other pituitary hormones as well (so-called "shotgun action"). For example, administration of large amounts of estrogen to young animals (rat, fowl) leads not only to atrophy of the gonads, but also to thyroid atrophy and to dwarfing.

Another mode of interaction between a trophic hormone and the dependent secondary hormone has been demonstrated in the case of thyrotrophin-thyroid interaction. In vitro, thyrotrophin is inactivated in the process of acting upon the thyroid gland. It can be reactivated in vitro, and possibly such inactivation and reactivation also occur in the living organism. This type of interaction has not been demonstrated for the other trophic pituitary hormones.

HYPOTHALAMIC CONTROL OF ANTERIOR PITUITARY. The pituitary stalk connects the pituitary gland with the hypothalamus. However, all of the nerve fibers in the stalk pass to the posterior lobe, none entering the anterior pituitary. An intricate and highly specialized vascular connection of the hypothalamus is present in the portal venous system of the stalk. It is possible that humoral "impulses" may be transmitted from the hypothalamus through this portal system to the anterior pituitary. The existence of stimulating substances thus transmitted has not been proven, but there are certain observations which are suggestive in this connection. Release of anterior pituitary luteinizing hormone can be accomplished in the rabbit by administration of epinephrine; the latter must be injected into the gland and does not cause release of hormone if injected intravenously. It probably is not the agent which causes the hormone release physiologically, but such experiments do show that adrenergic amines, acting upon the pituitary in sufficient concentration, can "fire" the gland. In other species, e.g., the rat, the pituitary appears to respond to cholinergic (acetylcholine) rather than to adrenergic stimulation.

The agent reaching the anterior pituitary from the hypothalamus would be expected to be produced or liberated in the nerve centers, and it would be quite possible that it then might reach the anterior pituitary in relatively high concentration. There is evidence that biogenic amines are present in, and probably produced by the central nervous system; one such amine has been partially purified (encephalin). It might be assumed that this, or a related substance, is liberated in the hypothalamus as a result of an afferent nerve impulse, and is transported to the anterior pituitary through the portal system of the stalk.

The ovulatory response of the rabbit has been studied extensively, but evidence that other hormones are released in a similar way is lacking. A failure of thyrotrophin release in the stalk-sectioned rat has been suggested.

Hypothalamic control of secretion of ACTH by the anterior pituitary has been demonstrated. Lesions of certain centers in the anterior hypothalamus prevent

ACTH release following stress (p. 427). The "impulse" from the hypothalamus does not reach the anterior pituitary through the stalk, inasmuch as stalk section does not impair normal pituitary response. It has been suggested that a hormonal agent liberated in the hypothalamic centers may reach the pituitary by way of the general circulation. The importance of the observations outlined above is that they permit us to visualize a mechanism whereby central nervous influences might be exerted upon the anterior pituitary, and thereby upon other endocrine glands. Certain clinical observations suggest that nervous influences may affect pituitary function in man; viz., psychogenic disturbances of the pituitary-ovarian cycle, precocious puberty following encephalitis and brain tumors, the release of ACTH (alarm reaction) following mental or emotional stress (p. 427), etc.

Gonadotrophic Hormones

The anterior pituitary secretes three hormones which exert important effects on the gonads and are therefore termed "gonadotrophic." These include: (1) follicle-stimulating hormone (FSH); (2) interstitial cell-stimulating hormone (ICSH) (luteinizing hormone; LH); (3) lactogenic hormone (prolactin) (luteotrophic hormone; LTH). In the female, they are responsible for the growth, maturation, and expulsion of the ova and the production of the internal secretions of the ovary. In the male, they stimulate spermatogenesis and production of androgen.

Hypophysectomy is followed by atrophy of the gonads, with abolition of their hormone production and, consequently, atrophy of the target organs of these hormones, viz., uterus, vaginal mucosa, prostate, and seminal vesicles. All of these structures and functions are restored by administration of the gonadotrophic hormones. Secretion of these pituitary factors is controlled by the levels of androgen and estrogen in the circulation, an increase in these steroid hormones causing depression and a decrease stimulation of gonadotrophin production.

Follicle-Stimulating Hormone. Follicle-stimulating hormone (FSH) is apparently a water-soluble glycoprotein, containing perhaps as much as 20 per cent glucose. FSH prepared from sheep pituitaries also contains glucosamine. Activity is destroyed by certain amylase preparations and by proteolytic enzymes.

ASSAY. Inasmuch as the activity of FSH is influenced by ICSH, quantitative data regarding the former can be obtained only in hypophysectomized animals. The following procedures may be employed: (1) production of increase of ovarian weight or follicular development of hypophysectomized female rats; (2) production of increase in weight of testes without stimulation of secondary sex organs in hypophysectomized male rats.

ACTIONS. In the female, FSH stimulates the growth and maturation of ovarian follicles and prepares them for ovulation. In pure form, this hormone does not cause secretion of estrogen by the ovary, but does so in the presence of even minute amounts of luteinizing hormone (ICSH; LH) (p. 402). In the male, FSH stimulates spermatogenesis.

Interstitial Cell-Stimulating Hormone. Interstitial cell-stimulating hormone (ICSH) (luteinizing hormone; LH) has been isolated in pure form. It is apparently a homogeneous, water-soluble, carbohydrate-containing (mannose; hexosamine) protein. ICSH prepared from different species exhibits differences in immunological specificity, chemical composition, isoelectric point, and molecular weight (sheep 40,000; swine 100,000). Activity is destroyed by certain proteolytic enzymes and by reduction of disulfide linkages.

Assay. ICSH activity may be assayed in several ways, the following being commonly employed: (1) increase of ovarian weight and corpora lutea production in normal immature rats primed by FSH; (2) repair of ovarian interstitial cells in hypophysectomized rats; (3) increase in weight of seminal vesicles in normal immature male rats; (4) increase in weight of the ventral lobe of the prostate in hypophysectomized male rats.

Actions. In the female, ICSH (LH) acts synergistically with FSH to cause ovulation of mature follicles and secretion of estrogen by the thecal and granulosa cells. It is concerned with corpus luteum formation and, in conjunction with luteotrophic (lactogenic) hormone (LTH), it is concerned with the production of estrogen and progesterone by the corpus luteum.

In the male, ICSH (LH) stimulates the development and functional activity of Leydig (interstitial) cells, and, consequently, the production of testicular androgen (p. 412). Its administration, therefore, produces effects in the organism (except on the testis) similar to those which follow administration of testosterone (p. 412).

Lactogenic Hormone. Lactogenic hormone (prolactin) (luteotrophic hormone; LTH) has been isolated in pure form. It is apparently a homogeneous carbohydrate-containing protein, with a molecular weight of about 26,500–32,000, relatively insoluble in water. Activity is destroyed by pepsin and trypsin.

Assay. The method usually employed depends upon the increase in weight of the crop sac of the pigeon after injection of lactogenic hormone. An international unit (I.U.) has been defined as "the specific activity contained in 0.1 mg. of the standard preparation (international standard)."

Actions. Lactogenic hormone (LTH) is responsible for lactation in the postpartum woman. It is also necessary for stimulation of functional activity (estrogen and progesterone production) of the corpus luteum. Its function in the male is unknown.

Chorionic Gonadotrophin. The placenta produces a gonadotrophic hormone which differs from the pituitary gonadotrophins but resembles ICSH (LH) in its biological actions. It reaches a maximum concentration in blood and urine usually in fifty to seventy days after the last previous normal menstrual period, this phenomenon constituting the basis for certain of the most valuable pregnancy tests (Tables 28, 29, pp. 385, 387).

The rising titer of chorionic gonadotrophin in the blood during pregnancy causes the persistence and continued function of the corpus luteum until the placental syncytiotrophoblast is capable of producing sufficient estrogen and progesterone to support the pregnancy.

Physiological Considerations. Although unquestionably gonadotrophins are secreted by the pituitary gland from the time of birth or earlier, detectable amounts do not appear normally in the urine until about ten to twelve years of age, being subsequently elaborated in increasing amount and producing gonadal stimulation and the phenomena of pubescence. In the female, after the menarche, the pituitary gonadotrophins are secreted in a cyclic fashion (Fig. 37). The follicle-stimulating type of gonadotrophin predominates during the first half of the menstrual cycle and the luteinizing during the last half, although there is evidence that both are present throughout the entire cycle. Shortly after, or possibly a few days before the onset of the menstrual flow, the gonadotrophin concentration (blood and urine) slowly rises. As the midcycle is approached, there is generally an increase in gonadotrophin concentration in the blood and urine. A sharp peak is commonly reached just before ovulation, which may be maintained for one to three days; in some women no such sudden rise can be observed, while in others more than one peak may occur between the seventh and twenty-first days of the cycle (Fig. 37 and Table 28). Under the influence of the rising concentration of gonadotrophin, one or more primordial follicles are stimulated to grow and to undergo maturation. As a rule, only one of the follicles ripens completely in preparation for ovulation. Apparently a proper synergistic mixture of the follicle-stimulating and luteinizing hormones must be present in order to furnish the necessary stimulus for normal ovulation. The prevalent belief is that ovulation, in the human, occurs about fourteen days before the next menstrual flow, regardless of the length of the cycle. Following ovulation, the corpus hemorrhagicum is converted to a corpus luteum under the influence of the luteinizing gonadotrophin. During the next ten days the corpus luteum grows and becomes more active functionally but, as the gonadotrophic activity diminishes in the premenstrual phase, this structure degenerates and becomes a corpus albicans unless pregnancy has supervened. Thus, under the influence of the gonadotrophic hormones of the hypophysis, the ovary has passed through two endocrine phases: (1) the growing follicle, which produces increasing amounts of estrogen and culminates in ovulation; (2) the corpus luteum, which produces both progesterone and estrogen, preparing the endometrium for pregnancy.

Pregnancy. During pregnancy the gonadotrophin content of the blood and urine increases enormously because of the production of huge amounts of chorionic gonadotrophin by the placenta. It is believed that the pituitary gonadotrophins are almost completely suppressed throughout the period of gestation. Chorionic gonadotrophin enters the maternal blood stream as soon as the chorionic villi have established contact with the maternal circulation, which usually occurs during the last week of the cycle. This increase in luteinizing factor causes the corpus luteum to persist and grow as the corpus luteum of pregnancy, which continues to function during the first trimester of pregnancy. Chorionic gonadotrophin rises rapidly (blood and urine) during the early weeks of gestation to reach a very high peak, usually between the sixth and twelfth weeks, falling off subsequently to more moderate levels at about the twentieth week, after which time the gonadotrophin concentration remains fairly constant for the remainder of the period of gestation (Figs. 38 and 39, Table 29). The early, rapid rise in gonadotrophic hormone has furnished the basis for a number of popular tests for

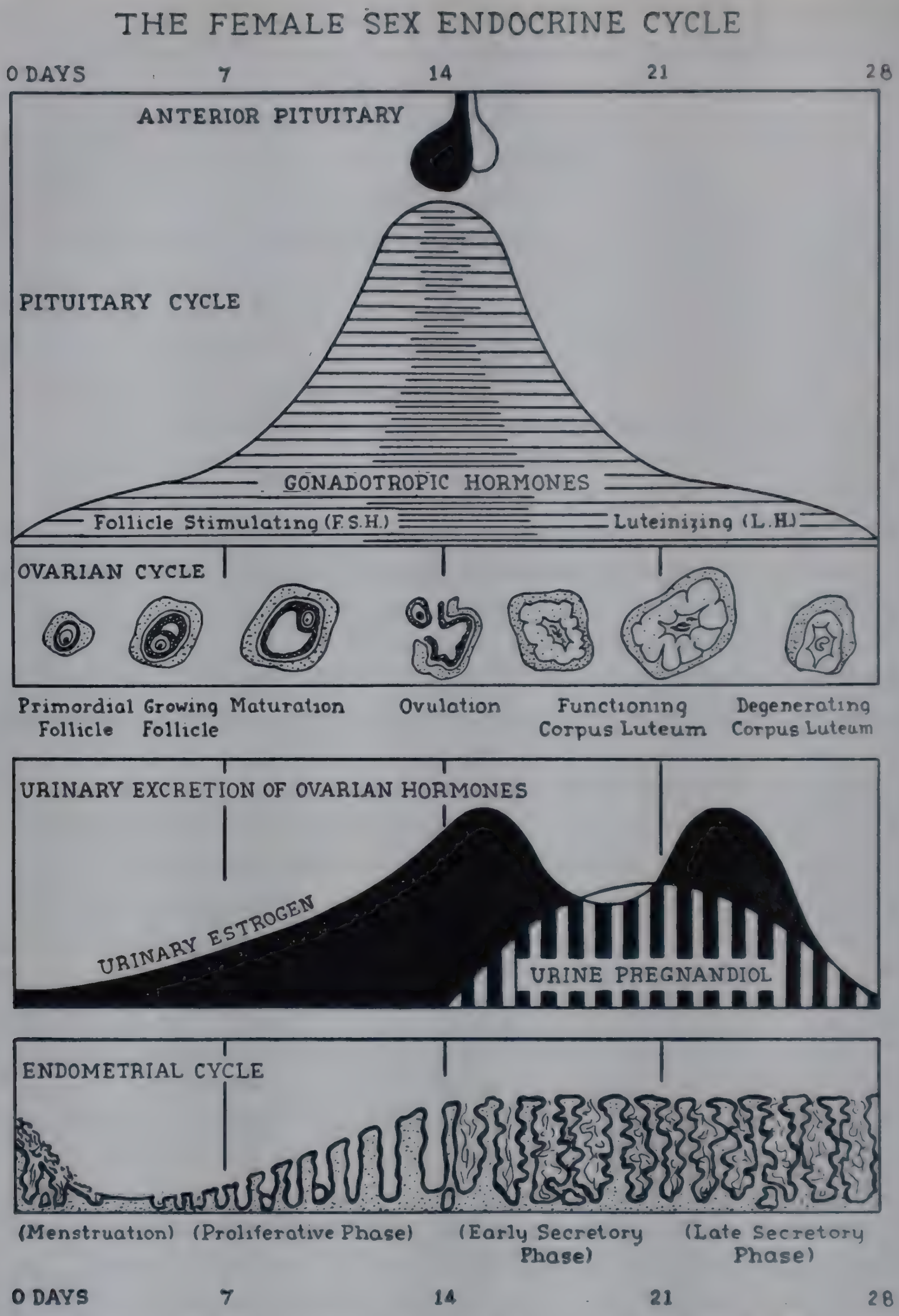
THE FEMALE SEX ENDOCRINE CYCLE
0 DAYS
7
14
21
28
ANTERIOR PITUITARY
PITUITARY CYCLE
GONADOTROPIC HORMONES
Follicle Stimulating (F.S.H.)
Luteinizing (L.H.)
OVARIAN CYCLE
Primordial Follicle
Growing Follicle
Maturation
Ovulation
Functioning Corpus Luteum
Degenerating Corpus Luteum
URINARY EXCRETION OF OVARIAN HORMONES
URINARY ESTROGEN
URINE PREGNANDIOL
ENDOMETRIAL CYCLE
(Menstruation)
(Proliferative Phase)
(Early Secretory Phase)
(Late Secretory Phase)
0 DAYS
7
14
21
28

Fig. 37. The female sex endocrine cycle (courtesy Dr. A. E. Rakoff.)

pregnancy, including the Friedman, Aschheim-Zondek, South African frog and the various rat ovary hyperemia tests.

The gonadotrophic hormone disappears rapidly from the blood and urine following delivery of the placenta, and normally is almost entirely absent within a week postpartum. It is believed that during the puerperium, lactogenic hormone (pituitary) blocks the liberation of pituitary gonadotrophins and thus results in a variable period of amenorrhea and low fertility during lactation.

Table 28. Sex Hormone Values in Urine and Serum

	FEMALE, DAYS OF THE MENSTRUAL CYCLE				MENOPAUSAL WOMEN	MALE
	7	14	21	28		
GONADOTROPHINS						
Urine[1]						
Range	Traces-12	8–40[6]	Traces-8	0–6	32–300	4-24
Average	6	20	6	Traces	80	8
Serum[2]	0-traces	Traces-3	0-traces	0	3–30	0
ESTROGENS						
Urine[3]						
Range	65–160	160–660[7]	160–660[7]	30–110	10–65	25–100
Average	110	330	500	65	Under 65	50
Serum[4]	Traces-6	3–9	3–9	0–3	0-traces	
PREGNANEDIOL						
Urine[5]						
Range	0	2–6	3–10	0–4	0	0
Average	0	4	6	Traces	0	0

[1] In mouse uterine-weight units per 24 hours.

[2] In mouse ovarian-weight units per 100 cc.

[3] In international units per 24 hours, assay made on castrate mice using vaginal smear method (1. m.u. approximately equivalent to 5 I.U.).

[4] In mouse units per 100 cc. complying with technic of Fluhmann.

[5] Mg. of sodium pregnanediol glucuronidate per 24 hours.

[6] Occasionally even higher peaks may be present, or occasionally more than one peak in one cycle.

[7] Two peaks in estrogen excretion are generally observed, one at the time of ovulation and another four or five days premenstrual.

MENOPAUSE. With the approach of the menopause, the ovary becomes progressively less responsive to gonadotrophic stimulation, with a consequent increasing functional demand upon the pituitary for gonadotrophic hormone, chiefly of the follicle-stimulating type. As estrogen production by the ovary continues to diminish, an increasing concentration of follicle-stimulating gonadotrophin appears in the blood and urine of the menopausal woman. The high gonadotrophin titer may persist for a variable number of years.

GONADOTROPHINS IN THE MALE. Gonadotrophins are usually demonstrable in the urine of boys about eleven to thirteen years of age, increasing gradually to reach a stable level by fourteen years of age. There is no cyclic rhythm in the urinary excretion of gonadotrophins, which are both follicle-stimulating and luteinizing in nature. A moderate increase in gonadotrophins occurs at times in men after the age of fifty years (male climacteric).

Determination of Gonadotrophic Hormones. When present in high concentration, as during pregnancy, gonadotrophins can be assayed by direct injection of blood or urine in suitable laboratory animals. In the normal, nonpregnant state, however, they are present in such small amounts that the urine or blood must be concentrated or extracted before the assay. The most commonly employed methods of gonadotrophin assay are based upon the effects of injection of urine, serum or partially purified extracts of these fluids into immature rodents. Several biologic end-points have been employed: (*a*) increase in weight of the uterus; (*b*) increase in weight of the ovaries; (*c*) production of corpora lutea; (*d*) vaginal estrus; (*e*) increase in weight of the seminal vesicles. The choice of method depends mainly upon the type and amount of gonadotrophin present. Results obtained by different laboratories must be interpreted on the basis of normal values obtained with the methods and technic employed.

Normal Gonadotrophin Values. CHILDHOOD. It is usually not possible to detect gonadotrophins in the urine before eleven years of age. In girls, it is often possible to detect small amounts of follicle-stimulating hormone as early as a year before the onset of the first menstruation, while in boys a positive response usually cannot be obtained before the age of twelve to thirteen years.

FEMALE REPRODUCTIVE PERIOD. Variable amounts of gonadotrophic hormone are excreted in the urine throughout most of the menstrual cycle in normal adult females. During the first week of the cycle the amount of hormone is usually quite small (traces to 8 mouse uterine-weight units). As the midcycle is approached, the titer suddenly rises and may reach a peak of 30 mouse uterine-weight units or more, falling suddenly subsequently. More than one such peak may appear during some cycles; it is not known whether this indicates the occurrence of more than one ovulation. Since such increases in excretion may occur quite suddenly, it is necessary to make assays at frequent intervals if this phenomenon is to be used as evidence of normal pituitary stimulation of ovulation. Gonadotrophic hormone may usually be demonstrated in whole blood or serum at the midcycle or during peaks of gonadotrophin excretion. However, because of the small amounts present, it is more practicable to employ urine extracts for clinical assay.

MALE REPRODUCTIVE PERIOD. Moderate amounts of gonadotrophic hormone (8–20 mouse units) may be regularly demonstrated in extracts of the urine of sexually mature males. The hormone appears to be chiefly of the follicle-stimulating type. Some degree of daily variation in gonadotrophin excretion has been noted, but there is no definite cyclic periodicity, such as occurs in women. The quantity of gonadotrophin in the blood of males is usually too small to be demonstrated by ordinary methods of clinical assay.

PREGNANCY. The normal values for gonadotrophic hormone in the blood and urine during pregnancy are indicated in Figures 38 and 39 and Table 29. It will be noted that the concentration of gonadotrophins in the serum and in the twenty-four-hour urine specimen are of approximately the same magnitude, although the urine exhibits greater day to day fluctuations.

As early as ten days after conception, an increase in gonadotrophins may be demonstrated in the blood and urine, and within ten days after the first missed period there is a sufficient concentration in the urine to give a positive "pregnancy test." By the sixth week of gestation values as high as 10,000 mouse

Table 29. Hormone Values in Normal Pregnancy

WEEKS	SERUM ESTROGENS (μg./100 ml.)*	SERUM GONADOTROPHIN (m.u./100 ml.)†	URINE PREGNANEDIOL (mg./24 hours)
2	1.5–8		2–10
4	1.5–10	50–1000	5–15
8	3–12.5	500–33000	5–15
12	4–16.5	1000–33000	8–20
16	8–20	1000–33000	8–30
20	16.5–25	330–10000	16–32
24	16.5–33	200–500	20–60
28	10–40	200–500	35–80
32	25–60	200–500	40–80
36	33–75	200–500	50–100
40	33–150	200–500	50–120

* As μg. estrone equivalents.
† Mouse ovarian units.

ovarian units per 100 cc. of blood serum (and urine) are commonly present and often a transient peak, up to 30,000 units, or even much higher in some cases, may be encountered between the sixth and twelfth weeks of gestation.

After the twentieth week the hormone level falls, and during the last half of the period of gestation the values remain quite constant, at from 300 to 500 mouse ovarian units per 100 cc. After delivery of the placenta, gonadotrophins fall rapidly, and within a week they have usually reached nonpregnant levels, although occasionally titers sufficient to give a positive "pregnancy test" may persist for several weeks, particularly if fragments of placenta have been retained.

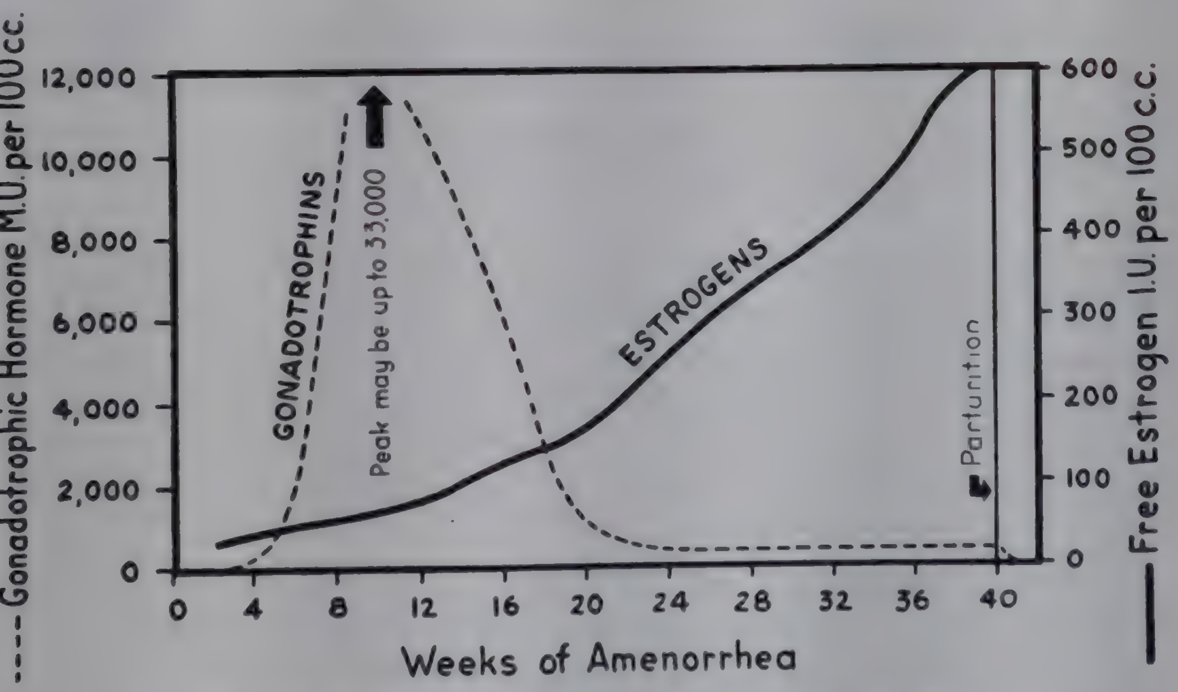

Fig. 38. Sex hormones in the blood during normal pregnancy.

Pregnancy Tests. The demonstration of increased amounts of gonadotrophin in the blood or urine constitutes the basis for the reliable tests for pregnancy. The two procedures that have stood the test of time are the Friedman and the Aschheim-Zondek tests, both of which are highly reliable when done properly. The South African frog (Xenopus laevis) also appears to be a reliable test animal, and has the advantage of giving rapid results, a positive response consisting in the extrusion of eggs within four to twelve hours after injection of serum or urine or their extracts. The South African frog has the added advantage of responding only to chorionic gonadotrophin, making the test more specific, but more data must be gathered before this method can be assumed to be as accurate as the Friedman or Aschheim-Zondek reactions. Present evidence indicates that it is not as sensitive as are the latter methods, so that false negative reactions may be obtained when the hormone titer is low. On the other hand, false positive reactions are rare. Another test for the rapid diagnosis of pregnancy is based upon the hyperemia produced in the ovaries of immature white rats in two to twenty-four hours following injection of pregnancy urine. The rat ovary hyperemia reactions are gaining popularity at present because of the rapidity and ease of performance of these procedures. However, interpretation of the end-point and certain points in technic require considerable experience in order to obtain a high degree of accuracy.

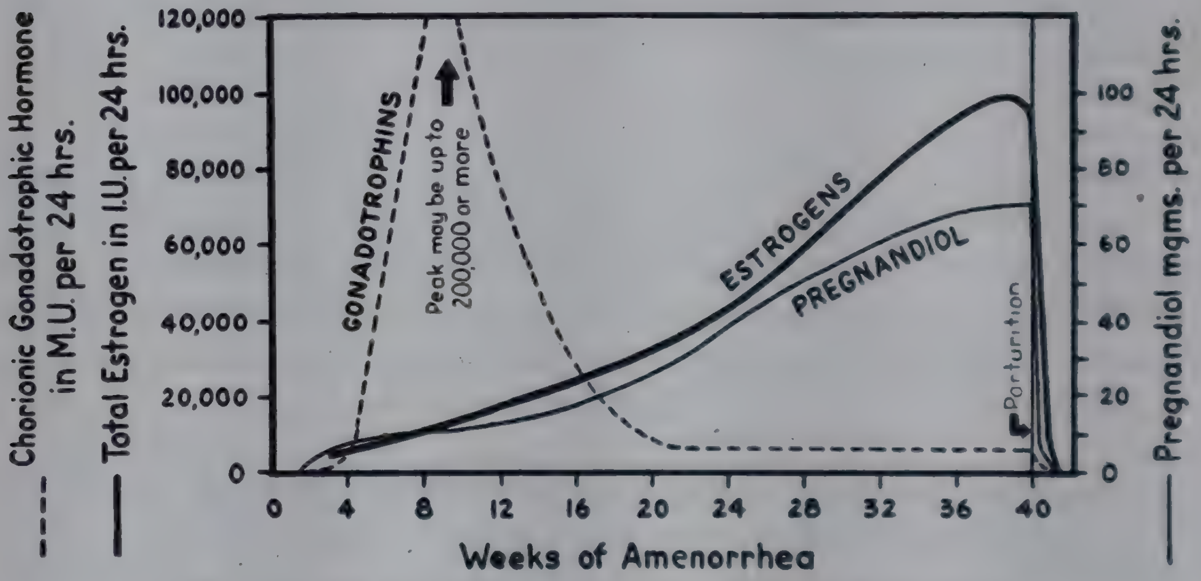

Fig. 39. Sex hormones in the urine during normal pregnancy.

Since these "pregnancy tests" indicate merely the presence of large amounts of gonadotrophin, it is apparent that they are not specifically diagnostic of pregnancy. Fortunately, there are but few other conditions in which gonadotrophic hormone is produced in amounts sufficient to give a positive reaction. Occasionally menopausal women will produce a sufficient amount of hormone to give a weakly positive reaction. If the test is repeated, using a smaller quantity of blood or urine, such false positives can usually be distinguished from pregnancy reactions. Chorionic tumors of all kinds, including hydatidiform mole, chorionepithelioma and chorionic tumors of the testes, frequently give positive results (p. 390).

It is frequently stated that positive tests are sometimes obtained in pituitary tumors and hyperthyroidism, but this is rare in our experience.

A false negative test for pregnancy may be obtained if the test is performed too early, within ten days after the first missed period, or if intrauterine fetal death has occurred. However, in the latter instance the pregnancy test may remain positive for several days to several weeks after death of the fetus.

Abnormal Gonadotrophin Values. The quantity of gonadotrophin in the blood and urine may be influenced by several factors. Increased amounts of hormone may be produced by the pituitary gland in various states of functional overactivity in which gonadotrophins may be produced (*a*) alone, in response to a specific stimulus, or (*b*) together with other pituitary hormones, when there is a state of general hyperactivity of the gland. Certain pituitary tumors may be accompanied by excessive gonadotrophin production, but this is by no means consistent or marked. Tumors of chorionic tissue, however, usually give rise to enormous increases in gonadotrophin of the luteinizing type. It is questionable whether any other type of tumor can produce gonadotrophic hormone, although this possibility has arisen in the case of other embryonal tumors, particularly of the testes.

The quantity of hormone in the blood and urine may be diminished in the presence of impaired pituitary activity, as a result of (*a*) a tumor involving the anterior hypophysis or neighboring structures, (*b*) states of functional panhypopituitarism, (*c*) specific functional deficiency in production of gonadotrophic hormones or (*d*) suppression of gonadotrophin formation by excessive amounts of gonadal hormones (estrogens, androgens). During pregnancy, there is a fall in gonadotrophins following expulsion of the placenta, in functional abnormalities of the placenta, and in intra-uterine fetal death.

INCREASE IN GONADOTROPHIC HORMONES. (1) *Pituitary Tumors.* Tumors of the anterior hypophysis seldom produce excessive amount of gonadotrophic hormones, and rarely is the increase marked; normal or subnormal values are usually obtained. Since these hormones are believed to be produced by the pituitary basophils, one would expect to find increased amounts in cases of pituitary basophilism (Cushing's syndrome); however, this occurs only sporadically, and usually early in the course of this disease. Later, gonadotrophins may not be demonstrable in the blood or urine.

(2) *Functional Hyperactivity of the Pituitary.* The production of increased amounts of gonadotrophic hormones by the pituitary usually results from increased functional demand for gonadal stimulation. At puberty, increased amounts are required to stimulate ovarian or testicular activity, but this increase is promptly checked by the consequent production of estrogens and androgens (push-pull theory), except in the presence of a primary ovarian or testicular deficiency, in which case increased gonadotrophic excretion (up to 300 mouse units per twenty-four hours) may persist for many years. Such deficiency of the gonads may result from agenesis, orchitis or oophoritis (particularly after mumps), failure of descent of the testes after puberty, cystic disease of the ovaries, trauma and castration. High urine gonadotrophin values are encountered in a syndrome

(Turner's syndrome) characterized by congenitally absent ovaries, with infantilism, short stature, and other congenital abnormalities, such as short, webbed neck, and coarctation of the aorta.

With the approach of the menopause, increasing amounts of gonadotrophins are produced in an effort to maintain function of the failing ovaries. This increased hypophyseal activity may persist for many years in some women, and is believed to be responsible for many of the symptoms of the menopause, particularly the hot flashes and general vasomotor instability. There is reason to believe that in some women there may be an associated overproduction of other pituitary factors, particularly the thyrotrophic and adrenocorticotrophic hormones. Increased amounts of gonadotrophins may appear within a week after removal of the ovaries and may remain at more than 100 mouse units for periods varying from six months to more than thirty years. The increase that follows "castration" by x-ray or radium is usually more gradual. Some women pass through the menopausal period with very little increase in gonadotrophins, and in occasional instances the hormone may fall to very low levels. In such cases the menopausal syndrome is likely to be atypical.

In the male, the increase in gonadotrophins at the time of the "climacteric" is not as consistent nor as marked as in the female.

Various menstrual disorders are frequently accompanied by increased excretion of gonadotrophins. In amenorrheic women, this indicates a primary ovarian deficiency. An increase is present in some cases of functional bleeding, particularly at puberty and at the menopause, being usually indicative of primary ovarian dysfunction. The persistent stimulation of abnormal ovaries by increased amounts of gonadotrophin may eventuate in cystic disease of the ovaries.

(3) *Increased Production by Chorionic Tissue.* By far the largest amounts of gonadotrophic hormone are encountered in the presence of growing chorionic tissue. The chorionic gonadotrophin is entirely of the luteinizing type, and, moreover, differs from pituitary luteinizing hormone (p. 381), although this distinction is not readily demonstrable by the usual methods of assay.

In normal pregnancy the hormone is demonstrable within ten days after the first missed period, in amounts sufficient to give positive biologic tests with small amounts of blood or urine, increasing enormously within six weeks (p. 388).

Hydatidiform mole, being a tumor of chorionic tissue, usually produces an even greater amount of gonadotrophic hormone than is observed in normal pregnancy (but usually no estrogen nor progesterone), up to several million mouse units per twenty-four hours in the urine, or 100,000 units per 100 cc. of serum. In cases in which such striking increases are not obtained, the diagnosis cannot be made readily by laboratory methods, because this condition usually occurs at about the end of the first trimester of pregnancy, at which time very high values are encountered normally.

Very high values are obtained also in chorionepithelioma, which is a condition of malignant degeneration of the chorion. Inasmuch as this condition follows expulsion of a mole, miscarriage, or normal pregnancy, the finding of progressively increasing amounts of gonadotrophin under such circumstances is highly significant, since the hormone titer should normally be diminishing rapidly. It should be emphasized, however, that a positive "pregnancy test" is not in itself diagnostically significant in this connection, since retained pieces of placental tissue or a missed abortion may occasionally produce a positive reaction weeks or even months following delivery. It has been claimed that in this condition chorionic gonadotrophin may be demonstrated also in the cerebrospinal fluid in titers up to 400 m.u. per liter; this has been suggested as a specific test for chorionepithelioma. In several proven cases we were unable to confirm this observation.

Increased quantities of gonadotrophins have been observed in the blood and urine in preeclampsia and eclampsia, often associated with decreased titers of estrogen and pregnanediol. In our experience, these findings are obtained in the majority, but not in all cases, nor does the severity of the toxemia necessarily parallel the increase in gonadotrophin. A similar increase occurs at times in some diabetic women during the last half of pregnancy. The significance of these observations is not known.

(4) *Tumors of the Testicle.* Certain malignant tumors of the testicle produce markedly increased amounts of gonadotrophic hormone, often enough to give a positive "pregnancy test." Values up to 300 mouse units per 100 cc. of serum (or up to 10,000 units per twenty-four hours' urine) are not rare. In the majority of instances these tumors are believed to be chorionepitheliomas, but because of existing difficulties in histologic classification there is some question as to whether other embryonal tumors may not produce gonadotrophic hormones. As a general principle, the largest amounts of hormone are produced by the more embryonal types of tumor. Metastases are often accompanied by marked increase in hormone concentration, while a decrease often follows removal or irradiation of the testicular tumor.

It must be emphasized that not all malignant tumors of the testis are associated with increased gonadotrophin values. Moreover, early in the course of development of those tumors that do produce gonadotrophin, the values may not be sufficiently high to give a positive reaction with the ordinary qualitative "pregnancy tests," and quantitative assay of the urine or blood may yield helpful information.

DECREASE IN GONADOTROPHIC HORMONE. (1) *Primary Pituitary Hypofunction.* The blood and urine gonadotrophins are diminished or absent in panhypopituitarism, as are all anterior pituitary hormones. They are absent in Simmonds' disease (pituitary cachexia), with consequent atrophy and functional failure of the gonads. Chromophobe tumors of the hypophysis are almost invariably accompanied by a diminution in gonadotrophic hormones. Basophilic (Cushing's syndrome) and eosinophilic (acromegaly) tumors may show some increase early in the course of their development, but later are usually associated with diminution in or absence of gonadotrophins.

The fact should be emphasized that many patients with obesity and hypogonadism, classified clinically as "hypopituitarism" or "Fröhlich's syndrome," may have normal or even excessive amounts of gonadotrophin, indicating that these are not true instances of hypopituitarism, but should be classified as primary

Table 30. Variations in Gonadotrophin Values

CONDITION	GONADOTROPHIN VALUES
Panhypopituitarism	
Simmonds' disease	Diminished or absent
Pituitary tumors	
Eosinophilic	Normal or increased early
Gigantism	Diminished later
Acromegaly	
Basophilic	Normal or increased early
Cushing's syndrome	Diminished later
Chromophobe	Usually diminished or absent
Hypogonadism	
Primary gonadal failure	
Castration	Increased
Atrophy, etc.	
Secondary (normal gonads)	Usually diminished, sometimes normal
Menopausal syndrome	Usually increased
Male climacteric	Usually increased, but not as consistently nor as markedly as in women
Testicular tumors	
Chorionepithelioma	Increased, often markedly
Other embryonal tumors	Moderate increase?
Interstitial cell tumors	
Seminomas	Usually normal
Functional menstrual disorders	Variable. Depends on etiology, ovarian function, etc.
Pregnancy	
Normal	Enormous increase. Varies with period of gestation (Figs. 38 and 39)
Hydatidiform mole	Usually increased
Chorionepithelioma	Progressive increase
Intra-uterine fetal death	Progressive decrease
Eclampsia and preeclampsia	Often increased
Diabetes	Sometimes increased late in pregnancy
Retained placental tissue	Delayed fall
Missed abortion	Delayed fall

hypogonadism. There is no consistent pattern of gonadotrophin excretion in patients with simple obesity, normal, decreased or increased values having been obtained in these cases.

In the female, diminished or irregular gonadotrophin excretion is commonly observed in association with infertility and with various menstrual disturbances, including amenorrhea, menorrhagia and other types of functional bleeding. Consistent and repeated failure to demonstrate adequate amounts of gonadotrophin in the urine of such patients is the only available method of establishing pituitary insufficiency as the underlying cause of the condition. This of the utmost importance in arriving at an etiologic diagnosis and in instituting rational therapy.

Diminished or irregular gonadotrophin excretion occurs much less frequently in the male than in the female. Diminished pituitary function is not a common cause of male infertility or impotence, which are much more often due to testicular dysfunction or to systemic causes. However, hypogonadotrophic (primary pituitary) eunuchoidism is by no means rare. Inasmuch as these cases respond at times to gonadotrophin therapy, differentiation between primary pituitary and primary testicular insufficiency as the basis for eunuchoidism is of considerable practical importance. This can usually be accomplished by urinary gonadotrophin assay in conjunction with testicular biopsy.

Malnutrition and various metabolic disorders may be followed by amenorrhea or other evidences of gonadal failure, due in some instances to diminished gonadotrophic activity. In our experience, the same mechanism may operate in cases of amenorrhea due to marked emotional disturbance, although the latter may also exert its influence in some cases directly on the gonad.

During pregnancy, a decrease in gonadotrophin titer below the normal range for that period of gestation usually indicates placental failure. In cases of intra-uterine fetal death or threatened miscarriage, the gonadotrophin content of the blood and urine usually falls progressively. It has been noted that when the serum gonadotrophin content falls below 100 mouse units per 100 cc., intra-uterine fetal death has occurred or may be expected, even though the usual pregnancy tests are still positive. The latter usually become negative in such cases after several days to several weeks. In ectopic pregnancy, the concentration of gonadotrophin is often below the level expected in normal gestation, probably because of poor implantation. The Friedman and Aschheim-Zondek tests may be negative in about 25 per cent of such cases, especially in the presence of tubal rupture or abortion.

(2) *Suppression of Gonadotrophin Production or Release.* Excessive amounts of estrogen, androgen and, possibly, progesterone may inhibit the production or release of gonadotrophins by the pituitary. This phenomenon may be observed clinically following administration of large amounts of these hormones. It occurs also during normal pregnancy, when the pituitary gonadotrophins are suppressed by the increasing level of estrogenic hormone. The same mechanism may be responsible for the low gonadotrophin values sometimes observed in patients with adrenocortical hyperfunction accompanied by high estrogen or androgen values.

Thyrotrophic (Thyroid-Stimulating) Hormone

Thyrotrophic hormone (TSH) has not been isolated in pure form, but rather highly purified preparations are available. It appears to be a protein, soluble in water, with a molecular weight of about 10,000. Activity is destroyed by pepsin, trypsin, and chymotrypsin.

Assay. Two sensitive methods have been described: (1) determination of the number of colloid droplets in the cells of the guinea pig thyroid following injection of preparations containing TSH; (2) measurement of increase in thyroid acinar cell height and extension of the hindlimb of the stasis (starved, non-metamorphosing) tadpole following injection of preparations containing TSH.

Actions. TSH is the most important factor in the regulation of thyroid function. Removal of TSH results in atrophy of the thyroid gland and cessation of thyroxine formation. Secretion of this hormone by the anterior pituitary is controlled by the level of circulating thyroxine, increase in the latter depressing and a decrease stimulating TSH production.

Injection of TSH is followed by morphological and metabolic evidences of increased thyroid function. The following phenomena have been observed: increase in thyroid acinar cell height; resorption of follicular colloid; increase of Golgi apparatus, proteolytic activity, oxidase granules, and O_2 consumption of thyroid cells; increase in the iodine content of the gland as well as in the "protein-bound" iodine of the plasma; increase in uptake of radioactive iodine by the thyroid. There is evidence that TSH is necessary for coupling of diiodotyrosine to form thyroxine (p. 267). Administration of TSH therefore produces effects on the organism (except on the thyroid) identical with those produced by thyroxine.

Abnormal Thyrotrophin (TSH) Secretion. Although procedures are available for assay of TSH in body fluids, they do not lend themselves to routine clinical use. There are relatively few data, therefore, on values for TSH in the blood or urine in clinical disorders. High values have been reported in acromegaly (p. 397), following thyroidectomy (p. 269), and after administration of various antithyroid agents (p. 269), as well as in some (but not all) cases of spontaneous hypothyroidism. Low values have been observed in panhypopituitarism.

Certain observations in experimental animals may perhaps be applicable clinically. Exposure to low environmental temperatures results in increased secretion of TSH, with consequent stimulation of thyroid activity. Conversely, secretion of TSH and thyroid hormone is decreased in a hot environment. These changes are important for adaptation, altering heat production to meet the different requirements imposed by these temperature changes. Other glands, especially the adrenal cortex, also play an important role in adaptation mechanisms.

TSH secretion diminishes during starvation in experimental animals. The thyroid gland presents a histologic picture suggesting decreased activity, which is reflected also in decrease in BMR.

Adrenocorticotrophic Hormone

Adrenocorticotrophic hormone (ACTH), as ordinarily available, is a protein with a molecular weight of about 20,000. However, there is evidence that all biological activity of this hormone resides in polypeptides with molecular weights of about 2500, which may contain as few as eight amino acids.

It has been suggested that there may be two adrenocorticotrophic hormones, concerned respectively with stimulation of adrenal secretion of (1) 11-17-oxycorticosteroids and (2) precursors of 17-ketosteroids. This does not appear to be the case in man.

Assay. Several methods of assay of ACTH activity have been proposed:

(*a*) REPAIR TEST. This consists in determination of the reappearance of lipid material in the regressed adrenal cortices of hypophysectomized rats following injection of preparations containing ACTH.

(*b*) MAINTENANCE TEST. This consists in determination of the quantity of ACTH-containing material required to maintain the adrenal weights of rats injected immediately after hypophysectomy.

(*c*) MEASUREMENT OF DECREASE IN ADRENAL ASCORBIC ACID. Measurement of the decrease in adrenal ascorbic acid of hypophysectomized rats one hour after intravenous injection of the ACTH-containing material is a highly specific and sensitive method.

Actions. ACTH is the most important physiological regulator of adrenal cortical activity. The adrenals of hypophysectomized animals are reduced in size and lipid content, and are restored to a normal state by injection of ACTH. Normal secretion of adrenal corticoids is also restored. Administration of ACTH to normal subjects causes increased secretion of adrenal steroids, with decrease (temporary) in adrenal lipid and ascorbic acid (pp. 426, 427), and practically all of the phenomena observed after administration of cortical hormones, including lymphopenia, eosinopenia, hyperglycemia, negative nitrogen balance, and electrolyte and water effects (p. 431ff.).

Release of ACTH by the anterior pituitary is apparently dependent in part upon a hormonal factor secreted by certain hypothalamic centers which can be stimulated by nerve impulses and by epinephrine. Its release may also be influenced by the level of cortical hormone in the tissues and/or circulation, an increased level depressing and a decreased level stimulating ACTH secretion.

Abnormal Adrenocorticotrophin (ACTH) Secretion. Satisfactory methods for assay of ACTH in body fluids have not been employed sufficiently extensively in man to provide much information regarding variations in this factor in clinical disorders. However, elevated blood ACTH levels have been observed in Addison's disease (untreated), the adrenogenital syndrome in children, and, according to some reports, in certain cases of myxedema, acromegaly, pheochromocytoma, and certain stress states (p. 428). The reports in Cushing's syndrome are contradictory; no increase has been found in the majority of cases. Increased urinary excretion of ACTH has been observed during the first twenty-four hours after surgical operations (p. 436).

Growth Hormone (Somatotropin)

Growth hormone has been isolated in apparently pure form. It is a protein, relatively insoluble in water, with a molecular weight of about 44,250, and an isoelectric point of pH 6.85. Activity is destroyed by pepsin and trypsin.

Assay. The most sensitive method consists in measurement of the increase in width of the proximal epiphyseal cartilage of the hypophysectomized female rat following injection of the growth hormone. Other methods that have been employed involve measurement of the increase in body weight of normal, plateaued or, better, hypophysectomized female rats following injection of the hormonal preparation.

Actions. Animals hypophysectomized before fully grown are dwarfed; administration of pituitary extracts results in gigantism or, if given after full growth is attained, in acromegaly. These effects on both skeletal and visceral growth are attributable to the growth hormone, but involve also participation of other factors, e.g., thyroxine and insulin, for their maximal expression. This is to be expected, inasmuch as the complicated phenomenon of growth must be dependent upon a number of interrelated metabolic processes and the state of nutrition of the organism.

Unfractionated extracts of the anterior pituitary produce effects on various metabolic processes, in part indirectly through the trophic hormones (e.g., TSH, ACTH), but also by a direct action not mediated by other glands. It is probable, but as yet not established with certainty, that most of these direct metabolic actions may be due to the growth hormone.

PROTEIN METABOLISM. Growth hormone is a protein-anabolic principle. It induces a positive nitrogen balance, accompanied by increase in tissue nitrogen, and decrease in urea nitrogen, amino nitrogen and total NPN of the blood plasma (p. 140). Insulin is necessary for production of this effect (p. 141).

CARBOHYDRATE METABOLISM. *In vitro,* crude pituitary extracts apparently inhibit the enzyme, hexokinase (p. 5), which catalyzes the phosphorylation of glucose to glucose-6-phosphate. Such extracts induce hyperglycemia and glycosuria, lower the respiratory quotient, and, if administered repeatedly, induce permanent diabetes in certain species (p. 70). This diabetogenic action of pituitary extracts in inhibiting carbohydrate utilization has been identified with the growth hormone.

The R.Q.-lowering effect, referred to above, appears to be due to interference with utilization of carbohydrate at some point between the formation of glucose-6-phosphate and of pyruvic acid, i.e., beyond the hexokinase reaction. There is some evidence, too, that growth hormone diminishes the uptake of glucose by tissues (isolated diaphragm), an effect that is counteracted by insulin.

LIPID METABOLISM. Administration of pituitary extracts stimulates mobilization of fat from the fat depots, with consequent decrease in carcass fat, and increase in plasma and liver lipids (p. 91) and in ketogenesis (p. 95). Increase in adrenal cortical hormone production is necessary for this fat-mobilization (p. 91). This phenomenon has been attributed to the growth hormone.

Excessive Growth Hormone Secretion. Hyperfunction of the eosinophilic cells of the anterior pituitary (usually eosinophilic adenoma) results in gigantism (longitudinal growth) if it occurs before the epiphyses have closed, and in acromegaly (appositional growth) if it occurs in older individuals. There is no clinically applicable method of assay of growth hormone. However, certain but not all of the manifestations of acromegaly can be explained on the basis of known metabolic actions of this factor. Variation at different periods is probably due to cyclic variations in secretory activity and to pressure effects of the growing tumor.

CARBOHYDRATE METABOLISM. Diminished glucose tolerance is demonstrable in the majority of cases of acromegaly at some time during the course of the disease (p. 47). Frank diabetes, i.e., fasting hyperglycemia and glycosuria, occurs in 15 to 20 per cent of cases. This is the clinical corrollary to diabetes produced by growth hormone in certain experimental animals (p. 70). In late stages, manifestations of hypopituitarism may supervene ("burned-out" pituitary gland). In such cases, the blood sugar tends to fall, and a state of "hypoglycemia unresponsiveness" may be revealed by the insulin tolerance (p. 53) or intravenous glucose tolerance (p. 51) tests.

MINERAL METABOLISM. Skeletal demineralization, with negative calcium and phosphorus balance, occurs in acromegaly. The serum Ca and alkaline phosphatase are usually within normal limits; the serum phosphate often tends to be elevated.

The basis for this phenomenon is not clear. It has been attributed to secondary states of hyperthyroidism, hyperparathyroidism, and hypogonadism, but none of these explanations appears to be entirely adequate.

BASAL METABOLISM. The thyroid is enlarged in 25 to 50 per cent of cases, and symptoms of thyrotoxicosis are not uncommon, accompanied by increase in BMR, although the histologic appearance of the gland usually does not suggest hyperactivity. An increased BMR, with or without symptoms of hyperthyroidism, suggests a state of active hypersecretion, but activity of the process is not precluded by the presence of a normal BMR.

High values for blood TSH (thyrotrophic hormone) have been reported in acromegalics, with normal blood levels of thyroxine. The possibility that at least in certain instances the elevated BMR may be due to extrathyroid factors is suggested by a normal or subnormal uptake of I^{131} by the enlarged thyroid in some acromegalics with increased BMR. On the other hand, in other cases, temporary improvement of thyrotoxic manifestations after iodine administration or subtotal thyroidectomy point to hyperactivity of the thyroid.

Decreased Growth Hormone Secretion. This is present characteristically in panhypopituitarism, in association with deficiency in adrenocorticotrophic, thyrotrophic, and gonadotrophic hormones. When it occurs in children (usually due to craniopharyngioma), deficiency in this factor is reflected in dwarfism. In adults, it may be due to Simmonds' disease (post-partum necrosis of pituitary), chromophobe adenomas of the pituitary, and suprasellar tumors encroaching upon the pituitary, e.g., craniopharyngiomas, gliomas, aneurysms, and meningiomas.

In such cases, as well as in pituitary dwarfism, metabolic abnormalities resulting from growth hormone deficiency (except dwarfism, in children) are overshadowed and obscured by the associated states of hypocorticoadrenalism, hypothyroidism, and hypogonadism. The diagnostic approach, from the standpoint of investigation of hormonal abnormalities, is directed to studies of deficiencies in function of the target organs of the trophic hormones (q.v.) rather than to the consequences of growth hormone deficiency.

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POSTERIOR PITUITARY HORMONES

Injection of an extract (pituitrin) of the posterior lobe of the pituitary (neurohypophysis) produces three well-recognized effects: (1) increase in blood pressure (pressor effect); (2) contraction of the mammalian uterus (oxytocic effect); (3) decreased urine volume in mammals, with increased concentration of urinary solids (antidiuretic effect). A fourth effect, induced in lower vertebrates, i.e., melanophore expansion or melanophore dispersion, is due to a principle formed in the pars intermedia, but present as a contaminant in unfractionated extracts of the posterior lobe. In animals that have no pars intermedia (e.g., whale, chicken), this principle occurs in extracts of the anterior lobe only.

The bulk of available evidence indicates that the pressor and antidiuretic effects are produced by the same substance (pressor principle). However, opinion is divided and evidence conflicting as to the chemical basis of the pressor and oxytocic actions. According to one view, the posterior lobe produces one substance with multiple activities; according to another, it produces at least two distinct substances.

A partially purified preparation, which evokes all of the above responses, exhibits characteristics of a protein with a molecular weight of about 30,000. Two fractions have been separated from such extracts, one (pitressin) producing a maximal pressor response, with little uterine effect, the other (pitocin) evoking maximal uterine contraction (oxytocic effect), with but little rise in blood pressure. Present evidence suggests that these pressor and oxytocic substances are polypeptides, with a molecular weight of about 2000. Pitocin (oxytocin) has recently been shown to be a cyclic octapeptide and has been synthesized, an accomplishment of historical importance. The synthesis of pitressin is currently being approached in a similar manner.

Assay. Pressor activity is assayed by comparing the rise in blood pressure, following intravenous injection in anesthesized dogs, spinal cats, or anesthetized rats, with that produced by known amounts of a standard preparation.

Oxytocic activity is assayed by comparing the degree of contraction of the isolated, virgin guinea pig uterus, in vitro, with that produced by known amounts of a standard preparation. Another method is based upon the blood pressure-lowering effect of the oxytocic principle in the anesthetized chicken.

Antidiuretic activity is assayed by comparing the reduction in urine volume following intravenous injection in hydrated unanesthetized rats or hydrated rabbits with that produced by a standard preparation.

Functions. Despite the pronounced pressor effect of pitressin, there is no satisfactory evidence that this factor plays a role in the physiological regulation of vascular tone or of blood pressure. Similarly, there is merely suggestive and contradictory evidence that the oxytocic principle may be implicated in the regulation of uterine contraction during parturition. On the other hand, there is unequivocal evidence that a hormone elaborated by the neurohypophysis, perhaps pitressin or a factor not yet separated from it (antidiuretic principle), is important in the regulation of water balance in mammals. This antidiuretic effect is dependent on a specific action of this factor on the epithelial cells of the distal portion of the uriniferous tubule, stimulating reabsorption of water independently of solids, and resulting in concentration of the urine (p. 566).

Secretion of this hormone is apparently regulated by nerve impulses originating in hypothalamic nuclei (e.g., supraoptic nuclei) and traversing the infundibular stalk (supraopticohypophyseal tract). It is believed that the activity of these hypothalamic nuclei is regulated by the degree of concentration of the blood plasma; i.e., a tendency toward reduction in plasma water content results in stimulation of antidiuresis with consequent renal tubular reabsorption of increased amounts of water. In the absence of this factor, the urine cannot be concentrated, and large volumes (up to 25 liters or more daily) of low specific gravity are excreted (diabetes insipidus). The presence of a functioning anterior pituitary is required for the development of this condition; i.e., total hypophysectomy does not result in diabetes insipidus.

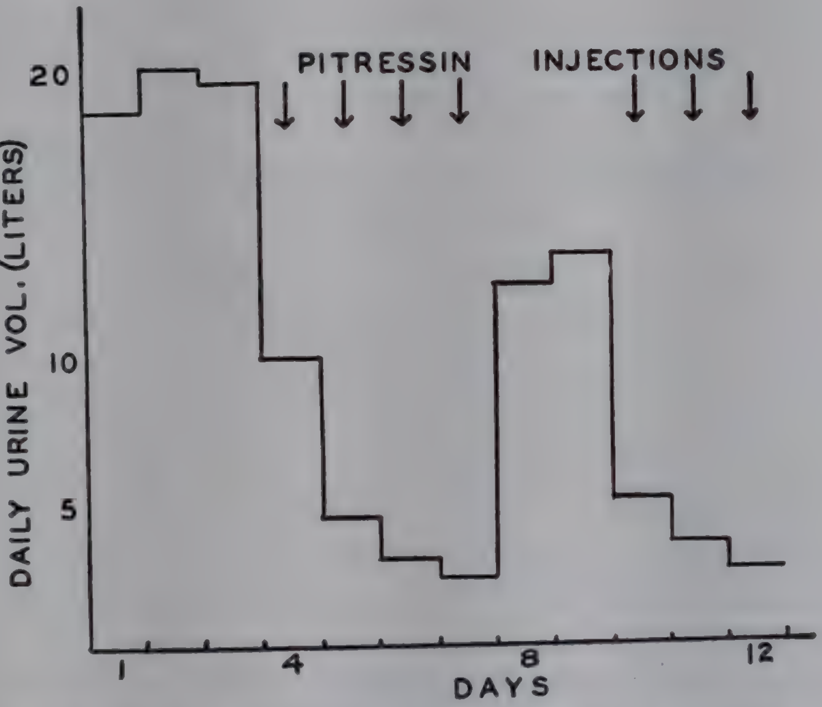


Fig. 40. The influence of administration of the antidiuretic hormone of the pituitary on the daily urine output of a patient with diabetes insipidus.

Injection of commercially available posterior lobe extracts commonly results in a rise in blood sugar. This action is not consistently identified with either the separated pressor or oxytocic fractions, suggesting that it is due to some nonspecific component of the extracts. In fact, highly purified preparations have no effect on the blood sugar of rabbits, and it appears highly improbable that the neurohypophysis plays a physiological role in the regulation of carbohydrate metabolism. A purported anti-insulin effect of posterior lobe extracts is also now regarded as a nonspecific property of these preparations.

The diagnosis of diabetes insipidus is suggested by the history of excessive fluid intake and output, but this condition must be differentiated from other causes of polyuria, e.g., diabetes mellitus, renal disease, and psychogenic polyuria and polydipsia. Differentiation from other organic diseases is usually easy, but that from psychogenic polydipsia is at times difficult. In the latter condition, however, if fluids are withheld (which for obvious reasons is difficult to enforce, and must be done under strictest supervision), the urine will be concentrated as in normal individuals, because there is no deficiency in antidiuretic hormone. Because of the difficulty of enforcing fluid restriction in individuals with polydipsia, the following procedure has been suggested, based on the fact that intravenous infusion of hypertonic solutions causes release of antidiuretic hormone from the neurohypophysis in normal individuals: In order to ensure an adequate urine flow, the subject is given 20 cc. of water per kg. of body weight over a period of one hour. An indwelling catheter is inserted thirty minutes after hydration is started, and urine is collected in fifteen-minute periods. The urine flow per minute is calculated. After two control periods of fifteen minutes each, 2.5 per cent sodium chloride solution is infused intravenously at the rate of 0.25 cc. per kg. per minute for forty-five minutes. In normal individuals and those with psychogenic polydipsia there is a sharp decrease of urine flow during, or within thirty minutes after the infusion. The subject with diabetes insipidus fails to show decreased urine flow but responds promptly to pitressin.

MELANOPHORE-EXPANDING (DISPERSING) HORMONE (INTERMEDIN)

Almost all cold-blooded vertebrates (e.g., amphibians, reptiles, fishes) are able to adapt their skin coloring to their surroundings to a greater or lesser extent, a protective or deceptive device of obvious benefit to the animal. This property is due to the presence, in the skin, of certain cells, with branching processes, termed "chromatophores," which contain mobile pigment granules. In the majority of such cells the pigment is black, and cells are called "melanophores"; xanthophores contain yellow, and erythrophores red pigment granules.

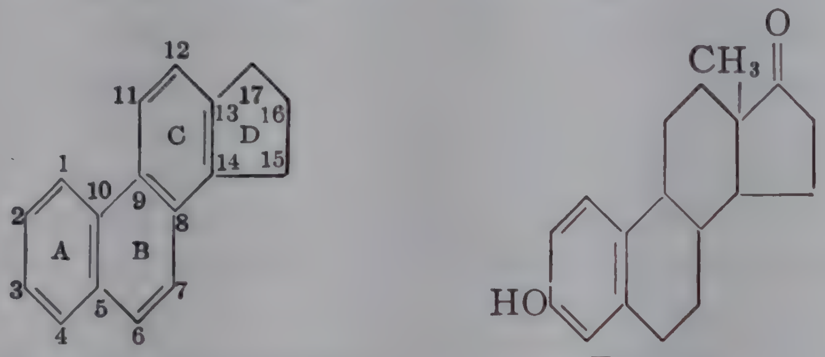
These pigment granules may be concentrated in a single mass near the center of the cell, in which case the skin becomes pale. They may, on the other hand, be dispersed throughout the cell and its processes, in which case the color of the skin is intensified. Scattering of these granules from their central position is effected by the action of the melanophore (chromatophore)-expanding (dispersing) hormone. In most species this is elaborated by the pars intermedia of the pituitary; in animals in which this structure is absent (e.g., whale, chicken), it is apparently produced in the anterior lobe. Variable amounts of this hormone are secreted, with consequent variations in skin color, in response to reflexes originating in the retina and initiated by the color of the immediate visible environment of the animal. Hypophysectomy (e.g., in the toad) results in paling of the skin; injection of the melanophore-dispersing principle restores the normal color. This forms the basis for the bioassay procedure for this hormone.

Little is known about the chemical nature of this substance. It has a molecular weight probably not much over 2000, is destroyed by tryptic but not by peptic digestion, is soluble in water, and is fairly heat stable.

STEROID HORMONES

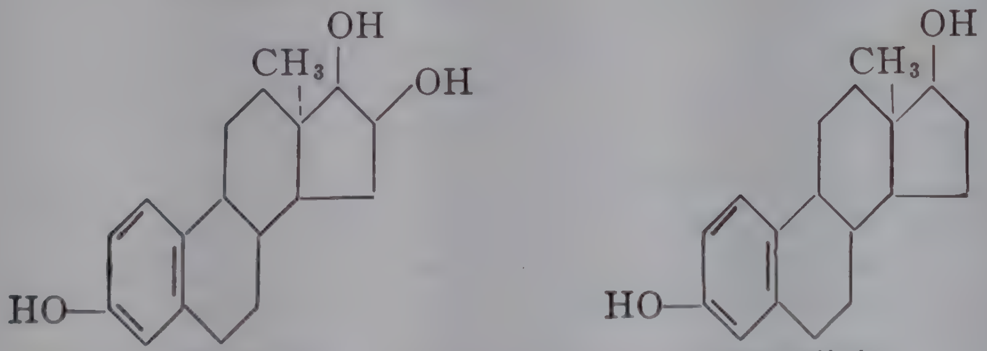
The term "steroid" is applied to the members of a group of compounds which have in common the cyclopentanoperhydrophenanthrene nucleus (Fig. 41). This skeleton structure consists of a cyclopentane ring, (D), fused to a completely hydrogenated phenanthrene (A-B-C). A carbon atom is present at every junction point in this ring system, its valence requirements (i.e., 4) being satisfied by H atoms. Substituents in the nucleus and on side chains are located by a standard system of numbering the carbon atoms, indicated in Figure 41.

The steroid hormones include the adrenal cortical hormones, estrogens, progesterone, and androgens. As indicated elsewhere (p. 84) they are related structurally, and perhaps metabolically, to cholesterol.



Cyclopentenophenanthrene Structure

Estrone



Estriol

Estradiol

Fig. 41. Structures of natural estrogens.

ESTROGENS

The naturally occurring estrogens in the human are estradiol, estrone, and estriol (Fig. 41). One of the essential features of these estrogens is the aromatic (unsaturated) character of ring A, and a hydroxyl (OH) group at C-3 position. They are soluble in oil and certain lipid solvents, but are only very slightly soluble in water.

Occurrence. Estradiol has been found in the placenta, in the urine during pregnancy, and in the testis of the stallion. Estrone is present in the urine of adult males, pregnant and nonpregnant women, pregnant mares, stallions, human placenta, the adrenal cortex, and stallion testes. Estriol is present in the urine of pregnant women and in human placenta. Other estrogens, viz., equilin, equilenine and hippulin, are present in pregnant mare urine.

Site of Formation. In the ovary, estrogens are produced by the maturing follicles (thecal and probably granulosa cells) and by the corpus luteum. All three pituitary gonadotrophic hormones (FSH, LH, LTH) are involved in stimulation of estrogen secretion by these structures (p. 381). Estrogen is also formed in the adrenal cortex (male and female) and in the placenta. In certain species at least (stallion), estrogen is apparently produced in the testis. There is some evidence that a small amount of estrogen may be formed from testosterone.

Metabolism. It is generally believed, although not proved, that estradiol is the primary estrogen formed by the ovary, and that cholesterol is a probable but not essential precursor. Estradiol and its metabolites circulate in the blood, partly in free form and partly conjugated, about 50 to 75 per cent being closely bound in some way to a plasma protein, probably β-globulin.

The liver plays an important role in the metabolism of estrogens. It contains enzyme systems which can convert estradiol in part to biologically inactive substances and in part to estrogens of lower activity, i.e., estrone and perhaps estriol. Conjugation of the estrogens is also believed to take place in the liver, with the formation and transfer to the blood stream of water-soluble glucuronidates and sulfates, which are ultimately excreted in the urine. Relatively large amounts of free estrogen are present in the bile of certain species, suggesting that biliary excretion may play an important part in the removal of estrogens from the circulation. Estrogens may subsequently undergo an enterohepatic circulation, being gradually degraded in the liver into compounds with no or lower estrogenic activity. There is evidence also that estrone may be converted to the more potent estradiol in this organ, a process of biological "activation." Undoubtedly, too, intermediary compounds are formed in the course of these metabolic changes which have not yet been identified.

The presumed functions of the liver in this connection may be summarized as follows: (1) it effectively removes estrogens from the systemic circulation by (a) biliary excretion and (b) metabolic degradation to less active and inactive compounds; (2) it conjugates them with glucuronic and sulfuric acids, converting them to water-soluble forms which return to the systemic circulation and are excreted in the urine; (3) it may convert estrone to the more potent estradiol.

Excretion. Estrogens are excreted in the urine, chiefly in conjugated form, as glucuronidates and sulfates of estriol, estrone, and estradiol. It is estimated that the urinary estrogens represent about 5 to 10 per cent of the activity of the estrogens secreted. The determination of urinary estrogens is therefore employed as an index of estrogen production, although variations in metabolic "inactivation" may conceivably interfere with accurate interpretation of such data. Normal values under different physiological conditions are presented in Tables 31, 32, and pages 405, 406.

Actions. Estrogens cause growth of the epithelium and musculature of the fallopian tubes, stimulate their contraction and motility, and are responsible for cyclic changes in the tubal muscosa. They cause growth, increased tonus and rhythmic contractions of the uterine musculature, and development of the endometrial mucosa and blood vessels which plays an important role in normal menstruation. They increase the vascularity of the cervix and stimulate secretion by the cervical glands, increasing the amount of cervical mucus with a lowered viscosity, thus favoring migration, motility, and longevity of spermatozoa. Estrogens cause a characteristic proliferation of the vaginal epithelium which forms the basis for one of the methods of bioassay of estrogenic activity (Allen-Doisy). They are also responsible in large part for normal development of the external female genitalia, the duct system of the breasts, and the nipples, and the secondary sex characteristics.

Administration of estrogens is followed by an increase in glycogen and alkaline phosphatase in the endometrium. Glycogen also increases in the vaginal epithelium, which is second only to the liver in glycogen content during the reproductive years (1.5–3%). The vaginal glycogen is probably the source of the lactic acid which, by increasing the acidity of the vaginal secretion (pH 4.0–5.0), favors a homogeneous flora of acid bacteria (Lactobacillus vaginae).

Apart from their actions on specific target tissues, indicated above, estrogens have but little effect on metabolic processes. They (particularly estradiol) exert a slight Na, Cl, and water-retaining effect, not nearly as pronounced, however, as that of the adrenocortical hormones, testosterone, or progesterone. They apparently favor retention and skeletal deposition of calcium. In birds, estrogens cause hyperlipemia; this does not occur in mammals, being related presumably to the influence of the ovarian hormones in the formation of the egg yolk.

Assay Procedures. Estrogens may be determined by bioassay of urinary extracts or of serum, by colorimetric or fluorometric methods, and by cytologic examination of vaginal smears.

VAGINAL CYTOLOGIC EXAMINATION. This is the simplest, most rapid, and least expensive method for estimating estrogenic activity. In many instances it is also the most satisfactory method for clinical purposes. Vaginal secretion may be aspirated from the posterior fornix of the vagina with a thick-walled glass pipette attached to a rubber bulb, blown and spread upon a slide, and before drying can occur, dropped into a jar or bottle containing equal parts of ether and 95 per cent alcohol. It may be kept in the fixative indefinitely until it is stained by the Papanicolaou or Shorr methods. For rapid use in the office or clinic, secretion can be collected from the lateral vaginal wall upon a cotton-tipped applicator, which is then dropped into a test tube containing 2 or 3 cc. of physiologic sodium chloride solution. A differential stain is then added; a drop is placed on a slide, covered with a slip, and examined immediately under the microscope.

With either the fixed or wet smear, at least six grades of estrogen function can be estimated, ranging from marked estrogen deficiency to marked estrogen effect. This method is usually adequate for determining the presence of ovarian deficiency or the relative state of function of the ovaries, for selecting the ovulatory phase, and for determining the response to estrogens in patients with ovarian deficiency. Its usefulness is limited in the presence of vaginal or cervical infections; the response is modified by progesterone or androgens. Quantitation of high estrogen values, above those which give a maximum estrogen effect, is not possible, nor of course is it possible to determine estrogen levels in the male by this method, although urethral smears have been suggested for this purpose.

VAGINAL SMEARS FOR GLYCOGEN CONTENT. The glycogen content of the vaginal epithelium is an index of estrogen effect. Dried or fixed smears treated with iodine solutions or vapor are satisfactory for roughly estimating an estrogen effect.

BIOASSAY. Urinary estrogens may be extracted with steroid solvents after initial acid hydrolysis. The estrogens may then be separated from the androgens by sodium hydroxide. After subsequent acidification, the estrogens are dissolved in a steroid solvent, the latter is evaporated, and the extract containing the estrogens is taken up in oil. The oily solution may be assayed for total estrogen content in castrate rats or mice by the Allen-Doisy method. The smallest amount which produces cornification of the vaginal epithelium is one rat unit. This is compared to a standard estrone solution. The bioassay may also be carried out by the more sensitive uterine weight method.

It is well recognized that the urinary estrogen determination is subject to error from a number of causes, including loss during hydrolysis, inadequate hydrolysis, difficulties in carrying out an adequate assay on a small number of animals, and variations in response due to the mixture of several different estrogens in the extract. For special purposes it is possible to separate the estradiol, estrone, and estriol fractions by their differences in solubility in sodium carbonate and benzene, after which each fraction may be assayed separately. This is not feasible or necessary for routine purposes.

Confusion often arises out of the expression of estrogenic activity in terms of mouse units (m.u.), rat units (r.u.) or international units (I.U.). Moreover, these units cannot be interconverted accurately if a mixture of estrogens is present. For estrone, which is the predominating estrogen in the urine of nonpregnant subjects, a rat unit is usually equivalent to 10 mouse units, while a mouse unit may equal 1 to 5 international units, depending on the method of assay. The following international standards have been established: (1) Estrone: one international unit (I.U.) is equivalent to 0.1 μg. (0.0001 mg.) crystalline estrone. (2) Estradiol benzoate: one international benzoate unit (I.B.U.) is equivalent to 0.1 μg.

COLORIMETRIC METHODS. Methods have been described for estimating estrogens in purified extracts by colorimetric methods. These technics are not highly sensitive nor specific; they are better suited to pregnancy urine, because of the high estrogen content, than to the urine of nonpregnant individuals.

FLUOROMETRIC METHODS. Technics are available which measure fluorescence excited by ultraviolet light rather than intensity of color. These methods are highly sensitive and permit determination of the small amounts of estrogen in normal urines. However, highly purified extracts are required because of the interference of other urinary constituents. Although fluorometric methods are in use at present in some laboratories, the time involved in preliminary purification of the urine extracts has limited their general use for routine clinical purposes.

Estrogens at Different Ages. The newborn infant is in a state of hyperestrogenism, the hormone being derived from the maternal circulation and disappearing (excretion and destruction) during the first ten days of extrauterine life. Small amounts of estrogen may be found in the urine during childhood; this is probably of adrenal origin, young male and female children excreting approximately equal amounts. As a rule, the blood and urine estrogen values in girls begin to increase significantly above those in boys at about 8 to 10 years of age. The slowly progressive increase rises sharply shortly before the menarche (Fig. 37, p. 384).

During the reproductive years, estrogen production, as gauged by urinary excretion values, follows a cyclic pattern. In the early follicular phase the titer is rather low; it rises progressively to an initial peak at about the time of ovulation, subsequently drops moderately for a few days, and increases again as the corpus luteum becomes well established. A second peak in estrogen production is generally reached at about the twenty-first to twenty-fourth day of the cycle, after which there is a rather abrupt drop during the immediately premenstrual phase. The estrogen curve is therefore biphasic, with two peaks, one at about the time of ovulation and the other at the height of corpus luteum activity. The first peak is usually but not invariably somewhat higher than that which occurs during the corpus luteum phase. It is probable, however, that more estrogen is produced during the corpus luteum phase than during the follicular phase (Fig. 37) (p. 384). It has been estimated that the ovary secretes about 10 mg. of estrogen, in terms of estrone, during a normal cycle.

Table 31. Normal Urine Estrogen Values (Fluorometric)

AGE (years)	URINE ESTROGEN (ug./24 hours)
5–10	3.0–6.5
31–37 (Men)	21.6–26.2
(Women)	10 –50
50–65 (Women)	0–23.4
Pregnancy (8–10 mos.)	365–1500

As the menopause is approached, urinary excretion of estrogens gradually diminishes and the cyclic fluctuations become more shallow. The titer continues to fall progressively in the postmenopausal years, although some estrogen may be found even in aged women. In old age, it is probable that estrogens are produced primarily by the adrenal cortex, but in some instances in which the quantity of estrogen has been greater than is usually encountered, persistent follicular activity has been presumed and has been supported by the finding of occasional functionally active follicles in the ovaries of old women. It has also been suggested that the interstitial cells of the ovary may be a source of estrogens in postmenopausal women.

Estrogens in Pregnancy. It is presumed that the increase in estrogen during the early weeks of pregnancy is practically entirely of corpus luteum origin and, indeed, it is probable that the placenta does not contribute appreciably to the secretion of estrogens until after about the sixtieth day. However, it is probable that by the one hundredth day the slowly rising titer of estrogen is almost entirely of placental origin. Estrogen values increase very slowly after conception and it is not until the twelfth to sixteenth weeks of amenorrhea that the values in urine and in serum are distinctly higher than the peak values in non-pregnant women. After the twentieth week the rise in estrogen is progressive until near term, at which time the values in the urine may reach levels of 50,000 to 100,000 international units or more in terms of biologic activity and about 50,000 μg. by colorimetric procedures per twenty-four hours. While there is also a progressive increase in the estrogen content of the blood, the concentration is at a very much lower level, with the peak level for serum estrogen at 1000 to 5000 international units per liter. In some instances a rather marked fall in urinary excretion of estrogens has been observed about one to two weeks before term, but other observers have found the rise to be continuous to the time of delivery.

Table 32. Estrogen Excretion During Normal Pregnancy

WEEKS PREGNANT	URINE PER 24-HOUR EXCRETION		
	Estrone μg.	Estradiol μg.	Estriol μg.
12	30	20	500
16	160	50	2000
20	400	60	4000
24	450	100	7000
28	900	60	10,000
32	400	60	10,000
36	400	50	20,000
39	450	60	30,000
39½	0	20	10,000

There has been considerable interest in the nature of the estrogens during pregnancy. The type of estrogen present in the blood is not known with certainty but there is some reason to believe that it may be chiefly estriol. In the blood, about 50 to 75 per cent of the estrogen is present in the free form while the remainder is in some way rather firmly bound to the serum protein.

The urinary estrogens are a mixture chiefly of estradiol, estrone, and estriol, the relative proportion of each varying at different phases of gestation (Table 32). Estriol increases rapidly as pregnancy progresses and, on a weight basis, accounts for more than 95 per cent of the estrogen excreted during the last half of gestation. Both estrone and estradiol increase until about the twenty-fifth week of gestation, after which time the estradiol level falls while the estrone values fluctuate. More than 99 per cent of the estrogen in pregnancy urine is in a conjugated state, chiefly as glucuronidate and sulfate. In the last two weeks of gestation a rapid conversion of combined to free estrogen has been reported, but not generally confirmed. After delivery of the placenta, estrogen is rapidly cleared from the blood and is usually not demonstrable after the third day. Urinary titers fall to nonpregnant levels within four to seven days.

Increased Estrogen Values

Increased Production. In true *precocious menarche,* gonadotrophin and estrogen values similar to those found in normally menstruating women may occur in association with normal manifestations of puberty. Such cases must be differentiated from precocious puberty due to granulosa-cell tumor or other neoplasms.

Very few tumors produce increased amounts of estrogen, certainly not quantities comparable to those formed late in pregnancy. *Granulosa-cell tumors, thecomas,* and *luteomas* of the ovary are usually accompanied by a moderate increase in the estrogen content of the blood and urine. This increase can often be detected in cases occurring in children before puberty and in women after the menopause. It is also evidenced by obvious estrogenic effects in the patient. The total urine estrogen may range from 50 to 400 m.u. per twenty-four hours. The diagnosis of granulosa-cell tumor is more difficult in women during the reproductive period because the urine estrogens may be within normal limits and the clinical estrogen effects are not obvious.

Increased amounts of estrogen may be found in some instances of *tumor or hyperplasia of the adrenal cortex.* Very high values (more than 500 I.U. per twenty-four hours) have been regarded by some as characteristic of adrenocortical carcinoma. However, this is by no means true of all cases of cortical carcinoma, so that negative findings cannot be regarded as significant in the diagnosis of this condition.

A moderate increase in estrogen excretion has occasionally been observed in cases of *testicular tumors* of chorionepithelioma origin, but normal findings are the rule in such cases.

Functional ovarian disorders are seldom accompanied by marked increase in the blood or twenty-four-hour urine estrogens. It is not unusual, however, to find a persistently high plateau of estrogen excretion, which may result in a state of hyperestrogenism. The monthly output of estrogens under such circumstances may be two to five times normal. This may be associated with various types of functional bleeding or amenorrhea, depending upon the pattern of estrogen excretion and the responsiveness of the uterus. Not uncommonly, it is associated with amenorrhea followed by prolonged periods of bleeding and a hyperplastic endometrium ("metropathia hemorrhagica").

Decreased Destruction. A considerable increase in the free estrogen content of the urine, with or without an increase in total estrogen excretion, has been observed in patients with *hepatic functional impairment* (cirrhosis, hepatitis). This has been attributed to diminished destruction or inactivation (or excretion) by the liver (p. 402). Such patients often exhibit clinical manifestations of hyperestrogenism, such as gynecomastia and testicular atrophy in the male, and menstrual disorders in the female. There is some evidence that malnutrition (protein and vitamin B deficiency) may interfere with hepatic inactivation of estrogens and thus contribute to the pathogenesis of various disorders such as menstrual dysfunction, chronic cystic mastitis, premenstrual tension, etc.

Decreased Estrogen Values

In women during the reproductive period, decreased estrogen values in the blood and urine may result either from (*a*) primary pituitary deficiency, with inadequate gonadotrophic stimulation of the ovaries, or from (*b*) a primary ovarian defect, despite normal or even excessive gonadotrophic stimulation. The distinction between primary and secondary ovarian (estrogen) deficiency can usually be made with certainty only by gonadotrophin assays.

Decreased estrogen values are found in Simmonds' disease and milder forms of panhypopituitarism, and in other conditions associated with diminished gonadotrophin production (p. 391). Primary estrogen deficiency in young girls may result in a state of sexual infantilism, amenorrhea, or hypomenorrhea. Relative estrogen deficiency or irregular estrogen production later in the reproductive period may result in a variety of disturbances, including secondary amenorrhea, hypomenorrhea, various types of functional bleeding, or sterility. A premature menopausal syndrome may develop if this ovarian deficiency persists. In patients with ovarian cysts and other benign lesions of the ovary, estrogen excretion may vary considerably, but is usually low.

Threatened miscarriage early in pregnancy is accompanied by marked reduction in estrogen and pregnanediol (p. 411). A marked fall in the estrogen level occurs regularly following death of the fetus at any stage of pregnancy. A moderate decrease in estrogen in the blood and urine has been observed in many patients with preeclampsia and eclampsia and also frequently in diabetic pregnant women. The latter condition (diabetes-pregnancy) is also accompanied often by a diminution in progesterone, which is believed by some to be responsible for the disturbance in estrogen metabolism. Fractionation of the estrogens in toxemic patients has shown that the estrone fraction drops to very low values or disappears entirely; the estriol fraction decreases considerably and the estradiol fraction increases.

Estrogen excretion is diminished in the male castrate, indicating that a portion of the estrogen in normal male urine is of testicular origin.

PROGESTERONE

Progesterone is a steroid hormone (Fig. 42), closely related structurally to the adrenal cortical hormones (p. 423). It is secreted by the corpus luteum of the ovary during the period of its functional activity. It appears suddenly on the day of ovulation or perhaps a day or two earlier, as indicated by the presence in the urine of its chief excretion product, pregnanediol. It is concerned, in the latter half of the menstrual cycle, mainly with preparation of the endometrium for nidation of the fertilized ovum if conception has occurred. During early pregnancy it is produced by the corpus luteum of pregnancy (stimulated by luteinizing and lactogenic hormones of the pituitary), and perhaps also by other ovarian luteinized cells, and later by the syncytial cells of the placenta. During gestation, progesterone, in conjunction with estrogen, appears to be concerned with maintenance of a state of quiescence of the uterine muscle.

Progesterone, or a closely related compound with progestational activity, is apparently formed also in the adrenal cortex (p. 426). Other adrenal steroids, such as desoxycorticosterone, may be metabolized to pregnanediol, the chief urinary metabolite of progesterone, which, however, has no progestational activity. The possibility that progesterone, or one of its precursors, may be produced in the testis, at least in certain species, is suggested by the facts (1) that the bull excretes large amounts of pregnanediol in the urine and (2) that pregnenolone, a possible precursor of progesterone, has been isolated from hog testes.

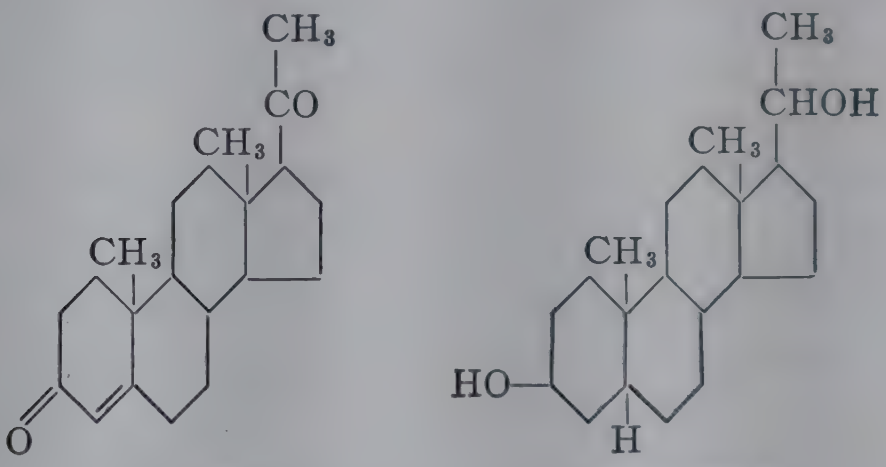
Metabolism. There is evidence that progesterone may originate from cholesterol, although the latter is not necessarily an immediate precursor. Acetate may be the only obligatory precursor. Comparatively little is known regarding its intermediary metabolism, although there is evidence that the liver plays an important role in this connection, as in the case of estrogens and androgens. Progesterone metabolites have been found in the bile; the liver appears to be involved in the removal of this hormone from the circulation, in its degradation to unidentified compounds, and in its reduction to pregnanediol, which is biologically inactive. The latter is then conjugated with glucuronic acid, passed into the systemic circulation, and excreted in the urine (Tables 28 and 29, pp. 385, 387). It has been estimated that 10 to 30 per cent of the progesterone secreted during a normal menstrual cycle is converted to pregnanediol.

Excretion. Human urine contains only biologically inactive progesterone metabolites, chiefly pregnanediol. Other isomers are present, especially during pregnancy, but in small amounts.

Actions. In the human being, progesterone produces characteristic changes (progestational) in the estrogen-primed endometrium. It also causes an increase in glycogen, mucin, and fat in the lining epithelial cells. Alkaline phosphatase decreases. Sudden decrease in progesterone and estrogen leads to a series of changes culminating in bleeding or menstruation. Progesterone modifies the action of estrogen on the vaginal epithelium during the menstrual cycle, causing desquamation and basophilia of the superficial layer of cells and leukocytic infiltration. In conjunction with estrogen, progesterone causes development of the alveolar system of the breasts and sensitizes them for the action of lactogenic hormone. It counteracts the effect of estrogen on the fallopian tubes and uterine cervix. In large doses it exerts androgenic effects, perhaps by conversion to androgenic metabolites.

Progesterone is responsible for the rise in basal temperature which occurs during the corpus luteum phase of the normal menstrual cycle. This is believed to be related to an increase in the basal metabolic rate. It favors retention of Na and water in the tissues (premenstrual fluid retention), its effect resembling, but not being as pronounced as that of the adrenal cortical steroids.

Methods of Assay. Progesterone may be assayed by the Corner-Allen method, in which a progestational effect is produced in the endometrium of castrated rabbits. Since only minute amounts of the hormone are present in the blood, even in pregnancy, a more sensitive method has been devised, in which the material to be assayed is introduced directly into the rabbit uterus and an endometrial response is obtained with relatively minute amounts of progesterone. These procedures are not practical for routine clinical use. More commonly, progesterone is determined indirectly by the quantitative determination of pregnanediol excretion in the urine.



Progesterone Pregnanediol

Fig. 42. Structures of progesterone and pregnanediol.

Pregnanediol is not found in the urine of normal children or men. In the normally menstruating woman, pregnanediol appears quite suddenly in the urine about twelve to fourteen days before the onset of menstruation, i.e., within a day or two after ovulation. The amount excreted daily in the urine varies, but usually ranges from 3 to 8 mg., occasionally reaching values as high as 11 or 12 mg. The total quantity excreted in the urine during a luteal phase may range from 20 to 80 mg. It usually reaches a peak about the twenty-first day of the menstrual cycle and then drops abruptly to zero two to three days before the onset of bleeding. The curve of pregnanediol excretion thus closely parallels the development, activity and regression of the corpus luteum (Fig. 37; Table 28).

If pregnancy occurs, the pregnanediol excretion does not diminish, but increases slightly until the tenth or twelfth week, and then continues to increase further to reach a peak of 60 to 100 mg. daily at or near term. At the time of onset of labor the rate of excretion may diminish, this drop being regarded as a factor in the mechanism of onset of labor (Fig. 39; Table 29). It has been suggested that the increase in excretion of pregnanediol early in pregnancy may serve as a basis for a satisfactory test for pregnancy.

Increased Pregnanediol Values

An increase in pregnanediol or pregnanediol-like substances may occur in cases of adrenal cortical hyperfunction, as in Cushing's syndrome, the adrenogenital syndrome, or tumors of the adrenal cortex not accompanied by clinical manifestations of these disorders, in both males and females. Values ranging from

2 to 25 mg. per twenty-four hours have been obtained in such cases. In these conditions the pregnanediol is an excretion product of steroids originating in the adrenal cortex.

Diminished Pregnanediol Values

Menstrual Disorders and Sterility. Absence of pregnanediol in the urine during the two weeks preceding menstruation is indicative of failure of ovulation and absence of a functionally active corpus luteum. In such instances the estrogen values are usually also diminished. There is still some question as to whether a normal pregnanediol excretion is conclusive evidence of the occurrence of ovulation. It should be pointed out that too much significance should not be attached to a single low value, because of the normally wide daily fluctuations. Furthermore, in studying patients with sterility in whom some defect in ovulation is suspected, it is well to correlate the pregnanediol findings with data obtained by endometrial biopsy, vaginal smears, basal temperature, etc. Absence of pregnanediol may be associated with anovulatory sterility, amenorrhea, and various types of functional bleeding. Patients with dysmenorrhea do not, as a rule, exhibit abnormal pregnanediol values, nor is there any specific relationship between pregnanediol and premenstrual tension, cystic mastitis, or various premenstrual disturbances.

Threatened and Habitual Abortion. The great majority of spontaneous abortions occur from the eighth to the twelfth weeks, during the period when the placenta is believed to be assuming the function of the corpus luteum of pregnancy. Inasmuch as the production of normal amounts of progesterone (and estrogen) is thought to be essential for the continuation of gestation, it is suspected that premature failure of the corpus luteum of pregnancy or inadequate placental secretion of progesterone may be the etiologic factor in many spontaneous abortions. Low pregnanediol and estrogen values have been found in patients who abort habitually during this period.

Intra-uterine Fetal Death. Diminishing pregnanediol titers are found regularly in the event of intra-uterine fetal death, although some days may elapse before low titers are reached.

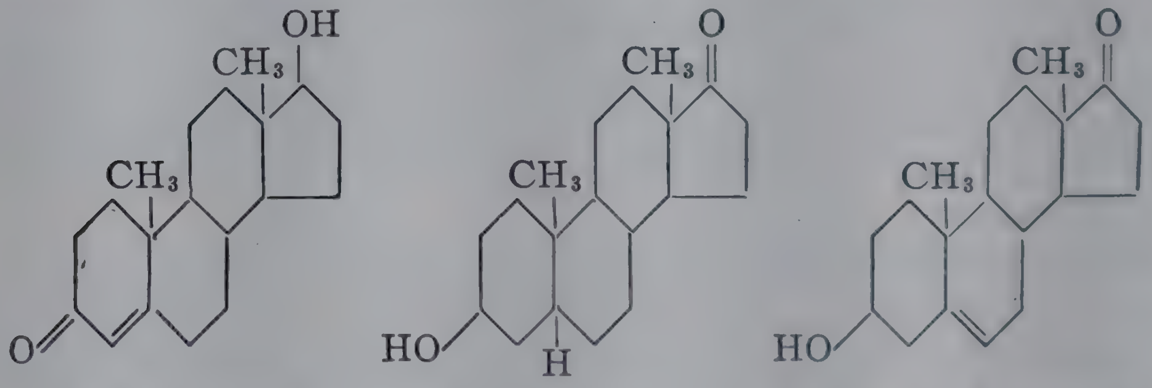
Toxemias of Late Pregnancy. There are a number of reports indicating that preeclampsia and eclampsia are accompanied by an abnormally low twenty-four-hour excretion of pregnanediol, as well as of estrogen (p. 408). The significance of this observation is not known. It is often associated with an increase in gonadotrophin (p. 391).

Diabetes in Pregnancy. A similar pattern of low pregnanediol and estrogen and high gonadotrophin values sometimes occurs in diabetic pregnant women, and has been employed as a criterion for instituting substitution therapy with estrogens and progesterone.

ANDROGENS

In addition to its spermatogenic function, the testis has an endocrine function, the production of male sex hormone, which is believed to be secreted in the form of testosterone. It is secreted by the interstitial cells of Leydig under the influence of pituitary gonadotrophic stimulation (interstitial-cell-stimulating gonadotrophin or luteinizing hormone). Male sex hormone is concerned with conditioning the mating drive and with maintaining the structure and function of the accessory reproductive organs, particularly the penis, prostate and seminal vesicles. It is also concerned with maintaining certain male characteristics, such as beard-growth and masculine hair distribution, deepening of the voice, male type of body development, etc. Testosterone is believed to play an important role in closure of the epiphyses in the growing male. Like the estrogens, androgens tend to inhibit the secretion or liberation of certain pituitary hormones, particularly gonadotrophins.

There is reason to believe that a portion (about two-thirds) of the androgenic substances that appear in the urine of males originates in the adrenal cortex. Androgens are present in the urine of women in amounts up to three-fourths of that in the male. It is possible that in women the hormone is derived from both the ovary and the adrenal cortex, although this is still open to question. The latter is generally believed to be the source of most, if not all, of the androgen in the female.



Testosterone Androsterone Dehydroandrosterone

Fig. 43. Structures of androgens.

The characteristic morphological effects of androgens are reflected in the changes induced in the male by castration. In the prepubertal castrate the prostate and seminal vesicles fail to develop; in adults, castration is followed by atrophy of these structures. The penis remains small in the former and regresses in the latter. A normal state is restored by administration of androgens. In prepubertal castrates, beard growth is absent, the body and pubic hair is scant and the voice remains high-pitched. Closure of epiphyseal lines is retarded, resulting in disproportionately long arms and legs.

Abnormally large amounts of androgen in women produce two types of effect, due to (1) "virilization" and (2) suppression of ovarian function. In the human being, the former includes: enlargement of the clitoris, growth of facial and body hair, development of a male forehead hairline, stimulation of secretion and proliferation of the skin sebaceous glands (often with acne), and deepening of the voice. The gonadal effects are due primarily to suppression of pituitary gonadotrophic function. This results in suppression of ovarian follicle maturation and ovulation (i.e., decreased estrogen and progesterone production) followed by atrophy of the uterus, vagina, and often the breasts.

Metabolism. Testosterone is not found normally in the urine. The following androgen metabolites have been found in the urine of normal untreated subjects (Fig. 43): (1) androsterone; (2) isoandrosterone; (3) dehydroisoandrosterone; (4) 11-hydroxyandrosterone; (5) androstenetriol; (6) etiocholanolone. The last two are biologically inactive and dehydroisoandrosterone is apparently derived largely from adrenal androgen (p. 425).

The urinary androgens occur chiefly as water-soluble sulfates and glucuronides which are biologically inactive (until hydrolyzed). The liver is believed to be the chief site of this conjugation, as well as of the conversion of testosterone to the above-mentioned metabolites, all of these changes tending to diminish biological activity (androgenicity). The water-solubility of the final conjugated products facilitates their removal from the body in the urine. In certain species, at least, androgens and their metabolites are in part excreted by the liver in the bile, and, like estrogens, they may undergo an enterohepatic circulation.

In vitro studies indicate that the liver contains enzyme systems that act on testosterone, converting it to androsterone (a 17-ketosteroid) and other metabolites. Tissues other than the liver may be involved, especially kidney. Following injection of testosterone, 15 to 60 per cent (average 35) of the amount administered can be accounted for by the recovery of 17-ketosteroids (mainly androsterone) in the urine. The nature of the metabolites of the remaining 40 to 85 per cent is not known.

There is some evidence that androgens may be converted in part to estrogens. The site of this conversion is uncertain.

Metabolic Actions. The dominant general metabolic effect of androgens is stimulation of protein anabolism. This is reflected in (1) a decrease in urinary nitrogen without an increase in blood NPN and (2) increase in body weight, due chiefly to an increase in skeletal muscle. In the growing organism, a growth spurt is induced, with increase in bone matrix and skeletal length. In addition to the specific stimulation of growth of the prostate and seminal vesicles, there is a rather selective increase in size and weight of the kidneys (renotropic action). This, together with the changes in renal enzymes and the absence of similar changes in the liver and intestine, has been interpreted as indicating that the protein anabolic effects of androgens are mediated, in part at least, by the kidney. Neither the pituitary growth hormone nor the adrenal cortex is involved in this phenomenon.

Creatine, which is virtually absent from the urine of normal men, increases after castration. This increase is abolished by testosterone, owing to increased storage of creatine in the muscles. After prolonged administration, the quantity of creatinine in the urine may increase, probably reflecting the increase in muscle mass.

Androgen reduces (and estrogen increases) the excretion of citrate in the urine. This is due apparently to increased reabsorption of citrate by the renal tubular epithelium.

The decreased urinary excretion of nitrogen (chiefly urea) that follow administration of androgens is accompanied by a lower urine volume and dimi ished excretion of Na, Cl, K, SO_4, and PO_4, with no increase in their concentra tions in the blood plasma. The tissue retention of K, SO_4, and PO_4 is probab related to the increased storage of protein. The retention of Na, Cl, and water apparently due to an action resembling that of the adrenocortical hormon (p. 432).

Another clinically important action of androgens is stimulation of formation acid phosphatase by the prostatic epithelial cells (p. 253).

Methods of Assay. Androgens are present in the blood, but not in sufficie concentration to be readily demonstrable by methods suitable for clinical pu poses. They are present in the urine in the form of water-soluble, biological inactive glucuronides, and can be obtained in a free, active state by acid hydroly followed by extraction with steroid solvents. After partial purification, the extracts can be assayed by biologic or colorimetric methods. Bioassay is tin consuming, expensive and difficult. The available colorimetric methods, a though they are nonspecific and do not yield results comparable to those obtain by bioassay (see 17-ketosteroids, p. 416), are the most valuable clinically a plicable procedures for studying androgenic function.

BIOLOGIC ASSAY. The most accurate method for bioassay of androgens based upon stimulation of comb-growth in the capon after administration extracts of the test material by injection or by direct application (inunction) the comb for a number of days. The results are expressed in terms of internatio units (I.U.), one international unit being equivalent to the activity of 0.1 mg. androsterone. Androsterone (major urinary metabolite) is only 10 to 20 p cent as active as testosterone, whereas dehydroisoandrosterone is only ab 25 per cent as active as androsterone. Methods based upon increased grow (weight) of the comb of the baby chick have also been described. Although as accurate as the capon method, satisfactory results have been obtained by t procedure when carefully controlled. The normal values obtained with the b chick method are about one-seventh those obtained by the capon method, testosterone assays about equally by these two methods. A correction factor m therefore be introduced when comparing results obtained with these procedur Tests based upon stimulation of the prostate or seminal vesicles of the castr rodent are not very satisfactory since other substances in the extracts may hav nonspecific augmenting effect.

Normal Values for Androgen (Bioassay by Capon Method). CH HOOD. Small amounts (up to 2 I.U. per liter) of androgenic activity may demonstrated in the urine of children of both sexes. Values of 3 to 20 I.U. obtained by colorimetric methods. A distinct rise occurs in boys soon after age of ten years, increasing gradually subsequently to the adult male level a puberty.

REPRODUCTIVE PERIOD. Normal men excrete an average of 70 I.U. androgen in twenty-four hours. Fluctuations from 30 to 100 I.U. occur commo but there is no definite cyclic variation.

Normal women excrete almost as much androgen as men, the daily values ually ranging from 30 to 100 I.U., with average values of about 50 I.U. wever, the androgen : estrogen ratio in the male is 2 to 5 times that in the nale. Although there are a few reports of cyclic variations in androgen excre-n in the female, the present consensus is that whereas there is considerable lly variation, this presents no definite pattern.

PREGNANCY. During normal pregnancy the androgen values generally fall thin the range for the normal nonpregnant woman, or may be lower.

CLIMACTERIC. Diminished androgen titers are frequently found in men after e age of fifty years. In menopausal or postmenopausal women, biologic androgen lues may be normal or diminished.

Table 33. Androgen (Biologic Assay)

	URINARY EXCRETION PER 24 HOURS IN I.U.	
	Range	Average
hildren (male and female) up to 10 years	0.5– 2.0	
oung adult males	30 –100	70
oung adult females	30 –100	50

bnormal Androgen Values. Consistently low values (3–40 I.U.) are tained in male hypogonads. Inasmuch as occasional low values may be obtained normal males, these should be checked by examination of more than one enty-four-hour specimen. The combination of subnormal androgen and high nadotrophin values is characteristic of primary testicular failure. This may occur eunuchs or as a sequel of orchitis, operations interfering with the blood supply the testes, systemic diseases, and the like. Diminution in or absence of onadotrophic hormone in men with subnormal androgen values suggests primary ituitary hypofunction. This may occur in panhypopituitarism, pituitary tumors nd true instances of Fröhlich's syndrome. A marked decrease occurs in patients ith Addison's disease, particularly in women. Severe nutritional disturbances ay also cause a decrease in androgens.

Functional disturbances in the pituitary-gonad cycle occur much less commonly the male than in the female. Moreover, disorders of spermatogenesis, with sociated infertility, are often not accompanied by any demonstrable abnormality f male sex hormone excretion.

Excessive excretion of biologically active androgens is encountered in associa-on with masculinizing tumors of the adrenal cortex, arrhenoblastomas of the ary and interstitial-cell tumors of the testis. In the last condition extremely gh values have been obtained, as much as the equivalent of 1000 mg. of drosterone per twenty-four hours, the hormone being present in the urine iefly as androsterone sulfate. Values up to 480 I.U. per twenty-four hours have been reported in cases of adrenal carcinoma. Some cases of adrenal adenoma or cancer have failed to show an increase in androgen, although a decrease was observed after removal of the tumor.

Increased androgen excretion occurs frequently in patients with adrenal cortical hyperplasia. The values in these cases are usually only moderately increased, but occasionally may reach as high as 500 capon units daily. A moderate increase is generally noted in Cushing's syndrome. It has been suggested that in cases of Cushing's syndrome in which the androgen values are normal, the disease is more likely to be primarily pituitary than adrenal in origin. Normal values are usually obtained in patients with simple hirsutism or virilism of constitutional origin.

17-KETOSTEROIDS

The term "17-ketosteroids" refers to those steroids possessing a ketone grou on the 17th carbon atom. They can be measured quantitatively, by the intensity of the red color that they produce with *m*-dinitrobenzene in alkaline solutio (Zimmerman reaction).

The principal 17-ketosteroids that have been isolated from normal human urin are (*a*) androsterone, (*b*) two of its isomers, isoandrosterone and etiochlolanol- alpha-17-one, (*c*) dehydroisoandrosterone and (*d*) estrone. Estrone, a phenoli substance, can be separated from the other 17-ketosteroids by treatment wit alkali. The remaining neutral 17-ketosteroids are all regarded as androstan derivatives (having 19 carbon atoms in the typical steroid skeleton) and have bee loosely called the urinary "androgens." They are believed to represent the excretory transformation products of certain adrenal and testicular hormones. Th quantity excreted in the male serves as an index of the combined steroid secretory activity of the adrenal cortex and the testis, and in the female chiefly of th adrenal cortex. Since some of the 17-ketosteroids are but weakly androgenic, o biologically inactive, the values obtained are not equivalent to those obtaine by bioassay. In most instances, however, the colorimetric and biologic valu roughly parallel each other.

The total 17-ketosteroids can be readily determined quantitatively in urine b means of the Zimmerman reaction, and also by a more specific colorimetr reaction (Pincus) which involves the development of a blue color with antimo trichloride. Since they are present in urine as esters, they must first be freed b careful hydrolysis; the free steroids are then removed by extraction with orga solvents and further purified to remove certain pigments and phenolic substance including the urinary estrogens. Further separation from nonketonic substances accomplished by treating with Girard's reagent.

The 17-ketosteroids can be further subdivided into alpha and beta fraction the beta-17-ketosteroids being precipitated by digitonin whereas the alpha a not. The alpha-17-ketosteroids normally predominate (85–95 per cent), co sisting of androsterone and etiocholanol-3 alpha-17-one, while the beta- ketosteroids are present in only small amounts and consist of isoandrosterone a dehydroisoandrosterone. These alpha and beta compounds are alcoholic nature; small amounts of nonalcoholic 17-ketosteroids may also be prese

In normal adults, alcoholic ketosteroids comprise 50 to 85 per cent and the beta-ketosteroids seldom more than 15 per cent and usually 2 to 3 per cent of the total (Table 34).

Normal Values for 17-Ketosteroids. The values to be given apply to the neutral 17-ketosteroids as determined (*a*) in the crude extract, corrected for interfering chromogens, or (*b*) in the purified ketone fraction. The values are expressed in terms of equivalents of androsterone.

Childhood. Children under six years of age usually excrete less than 1 mg. per twenty-four hours. The values then rise slowly to about the age of eighteen, although figures within the normal adult range may be encountered after the age of twelve years (Table 34).

*Table 34. Average Excretion of Alpha, Beta and Total Neutral Ketosteroids by Normal Subjects**

TYPE OF SUBJECT	NO. OF CASES	AVERAGE ALPHA NEUTRAL KETO-STEROIDS MG. PER 24 HOURS	BETA NEUTRAL KETOSTEROIDS, MG. PER 24 HR.		TOTAL NEUTRAL KETO-STEROIDS, MG. PER 24 HR.	
			Average	Range	Average	Range
Children						
4 to 7 years......	5	1.2	0.1	0.0–0.2	1.3	0.8–2.6
7 to 12 years.....	10	3.7	0.3	0.0–0.5	4.0	1.8–5.0
12 to 15 years.....	7	7.5	0.7	0.0–2.7	8.2	5.0–12.0
Women, 15+ years....	9	9.1	1.1	0.0–2.5	10.2	5.0–15.0
Men, 15+ years.....	5	13.8	1.2	0.0–1.8	15.0	8.0–20.0
Women, 4 to 7 months pregnant	4	13.2	1.9	0.0–4.0	15.1	10.0–20.0

* Talbot, N. B. et al.: New England J. Med., 223:369, 1940.

Adult Women. In normal adult women during the reproductive period, the 17-ketosteroid values usually range from 5 to 15 mg. per twenty-four hours. No cyclic fluctuations occur in association with the menstrual cycle.

Adult Men. In adult men, the usual average range is from 8 to 20 mg., although values up to 27 mg. daily have been regarded as normal. The higher values in men are attributable to the fact that the testes contribute to the total 17-ketosteroid output (about one-third of total amount), while the ovaries do not (excluding estrone), although the possibility of a difference in adrenal cortical function in the two sexes must be considered in this connection.

It has been observed that larger quantities of 17-ketosteroids are excreted during the night than during the day, but high night titers appear to be associated with high day titers, and vice versa. Day to day fluctuations occur commonly.

Pregnancy. The 17-ketosteroid excretion (excluding estrone) in pregnancy remains within the non-pregnant range, namely, 5 to 15 mg. per twenty-four hours, although values as high as 20 mg. may be obtained in late pregnancy.

Climacteric. Values ranging from 3 to 18 mg. per twenty-four hours may be obtained in menopausal women. As a rule, the values are normal or diminished moderately; moderately increased values are encountered occasionally, and are thought to be indicative of increased functional activity of the adrenal cortex. In men, the 17-ketosteroid values usually remain within the normal adult range up to the age of fifty years. Values below 5 mg. are commonly obtained in elderly men.

Decreased 17-Ketosteroid Values. The urinary 17-ketosteroids may be subnormal in primary or secondary hypofunction of the adrenal cortex or testis (interstitial cells). Low values may also occur in many primarily nonendocrine disorders in which the relation to adrenocortical or testicular function is not apparent. Such findings must therefore be evaluated critically.

Hypopituitarism. In panhypopituitarism, such as may be encountered in Simmonds' disease, certain brain tumors, pituitary infarction, and pituitary dwarfism, the urinary 17-ketosteroids are markedly diminished or absent (0–1 mg. per 24 hours). The absence of 17-ketosteroids in these conditions implies lack of stimulation of the adrenal cortex and gonads by the pituitary. Absence of 17-ketosteroids may be helpful in distinguishing true panhypopituitarism from conditions which may resemble it clinically, such as anorexia nervosa, in which the 17-ketosteroids are normal or diminished (but not absent).

In milder states of pituitary deficiency, particularly if not all of the trophic hormones are affected, the 17-ketosteroids may be low-normal or moderately diminished. It is not unusual to find them only moderately diminished in patients with hypogonadism with no gonadotrophins.

Pituitary Tumors. The 17-ketosteroids have been found to be normal or moderately diminished in patients with acromegaly, despite the fact that this condition is usually accompanied by hyperplasia of the adrenal cortex; it has therefore been suggested that the latter is compensatory in nature. Low, normal or moderately increased values may be obtained in pituitary basophilism at various stages of the disease. The very high values that may be encountered in some cases of Cushing's syndrome associated with carcinoma of the adrenal cortex are not usually obtained in cases due to primary pituitary disease. In chromophobe tumors of the pituitary, the 17-ketosteroids usually diminish rapidly and eventually disappear.

Male Hypogonadism. In hypogonad males, very low values (1–4 mg.) are more likely to be associated with primary pituitary hypofunction, whereas low-normal or moderately diminished values are encountered more often in primary hypogonadism. The gonadotrophins are usually high in the latter condition and low or absent in the former. These findings support the view that, in the male, only a fraction (probably about one-third) of the urinary 17-ketosteroids originates in the testis.

Female Hypogonadism. In young women with clinical evidence of ovarian deficiency, the 17-ketosteroids may vary as follows, depending upon the etiology and associated endocrine dysfunction:

(*a*) Absent or low, if due to panhypopituitarism.

(*b*) Moderately low if associated with various metabolic disorders, anorexia, or debilitating systemic disorders.

(*c*) Normal in the majority of patients with primary ovarian failure, since the neutral 17-ketosteroids are not of ovarian origin.

(*d*) Normal or moderately increased if associated with simple hirsutism or virilism, indicating possibly secondary hyperactivity of the adrenal cortex.

In patients with simple corpus luteum failure, as indicated by absence of pregnanediol in the urine and absence of secretory endometrium, the 17-ketosteroids are within normal limits.

ADRENOCORTICAL DEFICIENCY (ADDISON'S DISEASE) (p. 436). Women with Addison's disease excrete little or no 17-ketosteroid (0–1 mg. per 24 hours). In men with Addison's disease the values are markedly diminished, but rarely are these substances absent (1–4 mg.), since some testicular function is usually present. The determination of urinary 17-ketosteroids is a valuable laboratory aid in the diagnosis of this condition, especially in women.

HYPOTHYROIDISM. In the majority of patients with untreated hypothyroidism, the urinary 17-ketosteroids are diminished and sometimes very low, usually ranging from 0.5 to 3.0 mg. in myxedema. The values are generally normal but occasionally moderately low in hyperthyroidism.

HEPATIC DISEASE. The urinary 17-ketosteroids are often low in patients with acute or chronic liver disease, e.g., infectious hepatitis and cirrhosis. This is regarded as a consequence of diminished testicular hormonal activity resulting from lowered pituitary gonadotrophin secretion, due, in turn, to an increase in circulating estrogens (i.e., inefficient removal by liver, p. 402).

OTHER CONDITIONS. The 17-ketosteroids are often moderately low in chronic illnesses of all kinds, during the course of acute illnesses, including the common cold, in malnutrition, anemias, anorexia nervosa, malignancy, and after marked physical fatigue. Low values are commonly encountered in patients with nephritis and diabetes, bearing no relation, however, to the severity of the condition. In all of these chronic illnesses, the diminished values may be a reflection of diminished adrenal cortical function.

Increased 17-Ketosteroids. Very high values for total urinary 17-ketosteroids have been reported in adrenal cortical carcinoma, adrenal cortical hyperplasia, and interstitial-cell tumors of the testis. Ovarian arrhenoblastoma is associated with normal or moderately increased values. Clinical manifestations and fractionation of the 17-ketosteroids are important in the differential diagnosis of these disorders.

TESTICULAR TUMORS. Very high 17-ketosteroid values have been reported in a patient with an interstitial-cell tumor of the testis, in whom the urinary androgen was chiefly androsterone sulfate. Chorionepithelioma and other embryonic tumors of the testis are associated with normal or decreased 17-ketosteroid values.

ADRENAL HYPERFUNCTION (p. 434). Adrenal cortical hyperfunction may result in several clinical disorders, including Cushing's syndrome, the adrenogenital syndrome and a feminizing syndrome in males. These may be associated with simple hyperplasia or benign or malignant tumors of the adrenal cortex. The highest 17-ketosteroid values have been reported in patients with adrenal cortical carcinoma (up to 350 mg. or more per 24 hours), but there is considerable overlapping with cases of adrenal cortical hyperplasia. However, these two conditions may be distinguished in some but not all cases, by separating the urinary ketosteroids into alpha-alcoholic, beta-alcoholic and non-alcoholic fractions. The latter two (beta-alcoholic and non-alcoholic) are often greatly increased in adrenal cortical carcinoma, but are at times normal or only slightly increased in hyperplasia. This finding, of high total 17-ketosteroids, with relatively high beta-alcoholic and non-alcoholic fractions, has been observed in carcinoma of the adrenal cortex associated with an adrenogenital syndrome, in some, but not all cases of Cushing's syndrome, and in a male patient with abnormal feminization (p. 436).

In the few cases of arrhenoblastoma in which this determination has been made, the total 17-ketosteroid excretion has been normal or only slightly increased, despite the fact that this condition is accompanied by hirsutism and marked enlargement of the clitoris. It has been suggested that this tumor may secrete androgenic substances that are not excreted as 17-ketosteroids. An increase in 17-ketosteroids has been reported in cases of virilism associated with ovarian fibromatosis; this is attributed to adrenal hyperplasia.

Following Endocrine Therapy. Administration of chorionic gonadotrophin may be followed by an increase in urinary 17-ketosteroids in the hypogonad male due to stimulation of the testis, if the latter is capable of responding to stimulation. It has also been estimated that about 50 per cent of injected testosterone propionate is recoverable from the urine in 17-ketosteroid form. Administration of ACTH is followed by increased excretion of 17-ketosteroids in normal subjects and in those with adrenal hyperplasia, but often not in those with adrenal tumors (p. 434). Administration of cortisone is followed by a decrease in urinary 17-ketosteroids in normal subjects (depression of adrenal activity) but often not in patients with Cushing's syndrome (p. 434).

A slight increase occurs in a variety of conditions, such as acute illness, physical and emotional strain, starvation, anoxia, exposure to cold, injury, postoperative states, etc., as a reflection of the participation of the adrenal cortex in the "alarm reaction" (p. 427).

ADRENAL HORMONES

S

The mammalian adrenal gland consists of two embryologically and functionally independent portions, the medulla and the cortex. The adrenal medulla consists of chromaffin tissue and is essentially a portion of the autonomic nervous system. Although the hormones which it secretes have pronounced pharmacological effects, extirpation of all adrenal medullary tissue is followed by no important changes. The adrenal cortex, on the other hand, an epithelial structure, secretes hormones which are essential for life. Their most important actions are metabolic in nature, whereas those of the medullary hormones are exerted predominantly on unstriated muscle, vasomotor mechanisms, and the circulation.

Adrenal Medullary Hormones

Two biologically active compounds have been isolated from the adrenal medulla and synthesized, viz., epinephrine (adrenaline; adrenine) and norepinephrine (noradrenaline; Arterenol), the former being a methylated derivative of the latter. These substances are closely related to the amino acids, tyrosine and phenylalanine.

Extracts of the adrenal glands of cattle have been found to contain epinephrine and norepinephrine in the approximate proportions of 4 : 1. Relatively larger amounts of the latter have been found in adrenal medullary tumors.

The term "chromaffin tissue" has been applied to tissues in which the "chromaffin reaction" can be developed, i.e., oxidation of epinephrine and certain related compounds by bichromate or iodate, with the production of a colored compound.

In man, in addition to the adrenal medulla, substances giving this "chromaffin" reaction are present in cells of the paraganglia and various parts of the sympathetic nervous system. The material in these situations, designated "sympathin," closely resembles epinephrine and norepinephrine, apparently the latter in most instances. The amounts of these substances increase after sympathetic stimulation.

Regulation of Epinephrine Secretion. Epinephrine is secreted by the adrenal medulla in response to stimulation of nerve fibers in the thoracolumbar portion of the spinal cord (splanchnics). This stimulation may be accomplished by a variety of factors, including fear, anger, pain, and other undesirable emotional states, hypoglycemia, hemorrhage, muscular activity, and anesthetic and hypnotic drugs. There is evidence that this mechanism is involved in the initiation of the "alarm reaction" (p. 427), the discharged epinephrine stimulating secretion of ACTH by the anterior pituitary, perhaps indirectly through mediation by a hypothalamic mechanism.

Biosynthesis and Metabolism of Epinephrine. The mechanism of formation of epinephrine is not known. Moreover, it is not certain that all of the steps involved take place in the adrenal medulla. Tyrosine or phenylalanine are generally regarded as the primary precursors, and conversion of phenylalanine to both tyrosine and epinephrine has been demonstrated by radioactive tracer technics.

Stimulation of sympathetic nerves is accompanied by liberation of a "neurohormone" with sympathomimetic properties, which has been identified in some instances as norepinephrine (Arterenol) and in others as epinephrine. It is possible that the former may be an immediate precursor of the latter, the final process of N-methylation required for this conversion occurring in certain tissues (e.g., the adrenal medulla) but not in others.

Epinephrine and norepinephrine disappear rapidly following injection. Little is known about possible excreted metabolites. Oxidative deamination by a monamine oxidase, an enzyme which is widely distributed in mammalian tissues, is apparently an important mechanism of inactivation of these hormones.

Metabolic Actions. Injection of epinephrine is followed promptly by an increase in blood sugar and lactic acid concentrations, due to acceleration of glycogenolysis in the liver (glucose) and glycolysis in muscles (lactic acid), associated with stimulation of phosphorylase activity (reactivation of inactivated phosphorylase). The hyperglycemic effect of norepinephrine is about one-twentieth that of epinephrine. This mobilization of glycogen is the major metabolic effect of epinephrine. In the hepatectomized animal, this hormone causes an increase in blood lactic acid but not in glucose. The acceleration of glycogenolysis is accompanied by accumulation of hexosephosphate, at least in muscle, and a simultaneous fall in the plasma inorganic phosphate concentration.

Utilization of carbohydrate is apparently depressed somewhat, although the the respiratory quotient increases transitorily. A simultaneous increase in blood ketone acids and the increase in lactic acid produce a decrease in blood bicarbonate. The oxygen consumption (basal metabolism) increases, according to some representing a specific calorigenic effect and, according to others, merely reflecting general sympathetic stimulation and increased muscle tonus.

Assay. These substances may be assayed by biological, chemical, chromatographic, and fluorometric methods. The fluorometric procedures are perhaps the most accurate.

The blood plasma of normal subjects contains about 0.2 to 0.6 μg. of these hormones per 100 cc., 60 to 80 per cent of which is norepinephrine, which, however, comprises only 15 per cent of the hormone content of the adrenal medulla. The urinary excretion is about 1 to 2 μg. per hour.

Adrenal Medullary Hyperfunction. This occurs most typically in the presence of pheochromocytomas (chromaffinomas). The clinical course of this condition is characterized by paroxysmal attacks due to sudden liberation of excessive amounts of epinephrine and/or norepinephrine by the tumor. Aside from the clinical manifestations, the most definitive diagnostic feature is an increase in the amounts of these hormones in the blood plasma and/or urine. The studies must be made during or very shortly after an attack. Plasma levels as high as 4 μg./100 cc. have been observed; values above 1 μg./100 cc. are highly suggestive, in the presence of the usual clinical features. Urinary excretion values as high as 100 μg. per hour have been reported; values over 100 μg. per day are highly suggestive of this disease. In cases of intra-adrenal medullary tumors, the increase is likely to be predominantly in epinephrine. A predominance of norepinephrine suggests an extra-adrenal tumor (sympathetic trunk).

Increased urinary excretion of epinephrine and norepinephrine occurs also during strenuous exercise, in insulin hypoglycemia, acute coronary occlusion (mainly norepinephrine), and other stress states (mainly epinephrine.)

METABOLIC MANIFESTATIONS. The blood sugar during the attacks is often, but not always, found to be abnormally high. The blood sugar concentration in intervals between attacks is usually normal. In some instances the glucose tolerance is impaired, the curve showing an abnormally high rise and/or a prolonged hyperglycemic plateau. These patients may maintain normal blood sugar levels and remain aglycosuric without insulin and without dietary restriction, but the persistent latent diabetes may be revealed by glucose tolerance tests. Several cases of diabetes associated with pheochromocytoma have been observed. High insulin requirement of this type of diabetes has been recorded. After extirpation of the tumor the diabetes improves. The diabetes may possibly be caused in these cases by continued epinephrine-induced hyperglycemia and secondary exhaustion and degeneration of the Langerhans islets (p. 69). In other cases there is no demonstrable impairment of carbohydrate metabolism in intervals between paroxysms.

The basal metabolism is high in some, but not in all cases of pheochromocytoma; this, too, appears to be the result of excess circulating epinephrine (p. 374).

OTHER DIAGNOSTIC PROCEDURES. Several tests have been proposed which depend upon the blood-pressure response to certain agents. Such substances as histamine, tetraethylammonium salts, and acetyl-β-methylcholine, which lower blood pressure in normal subjects, evoke a hypertensive response in those with pheochromocytoma, due presumably to reactive stimulation of hypersecretion of epinephrine and norepinephrine.

Another type of test procedure consists in the administration of certain adrenolytic drugs (e.g., benzodioxane, dibenamine). These agents counteract the vasopressor action of epinephrine and, therefore, produce a fall in blood pressure in cases of sustained hypertension due to pheochromocytoma.

The general experience has been that false positive and false negative results are obtained with both types of tests, although they may be extremely useful in certain instances.

Adrenal Cortical Hormones

The adrenal cortex produces a number of steroid hormones which influence many physiologic functions, including electrolyte and water balance, capillary permeability, carbohydrate, protein and lipid metabolism, resistance to stress and toxins, growth, lactation, pigmentation, and gonadal and renal function. The adrenal cortex is stimulated, morphologically and functionally, by the corticotrophic hormone of the anterior hypophysis. About thirty steroid substances have been isolated from the adrenal cortex in pure crystalline form; certain of these have been found to be active, either in prolonging life in adrenalectomized animals or in preventing or relieving individual manifestations of deficiency. The biologically active compounds isolated from the adrenal cortex may be classified as follows:

1. Adrenal cortical hormones proper ("corticoids").
2. Androgenic 17-ketosteroids.
3. Estrogens.
4. Progesterone.

Corticoids. Three structural features are essential for all known biological actions of these adrenocortical hormones: (1) a double bond at C-4-5, (2) a ketonic group (C=O) at C-3 (i.e., an α, β-unsaturated ketone group), and (3) a ketonic group at C-20 (Fig. 44).

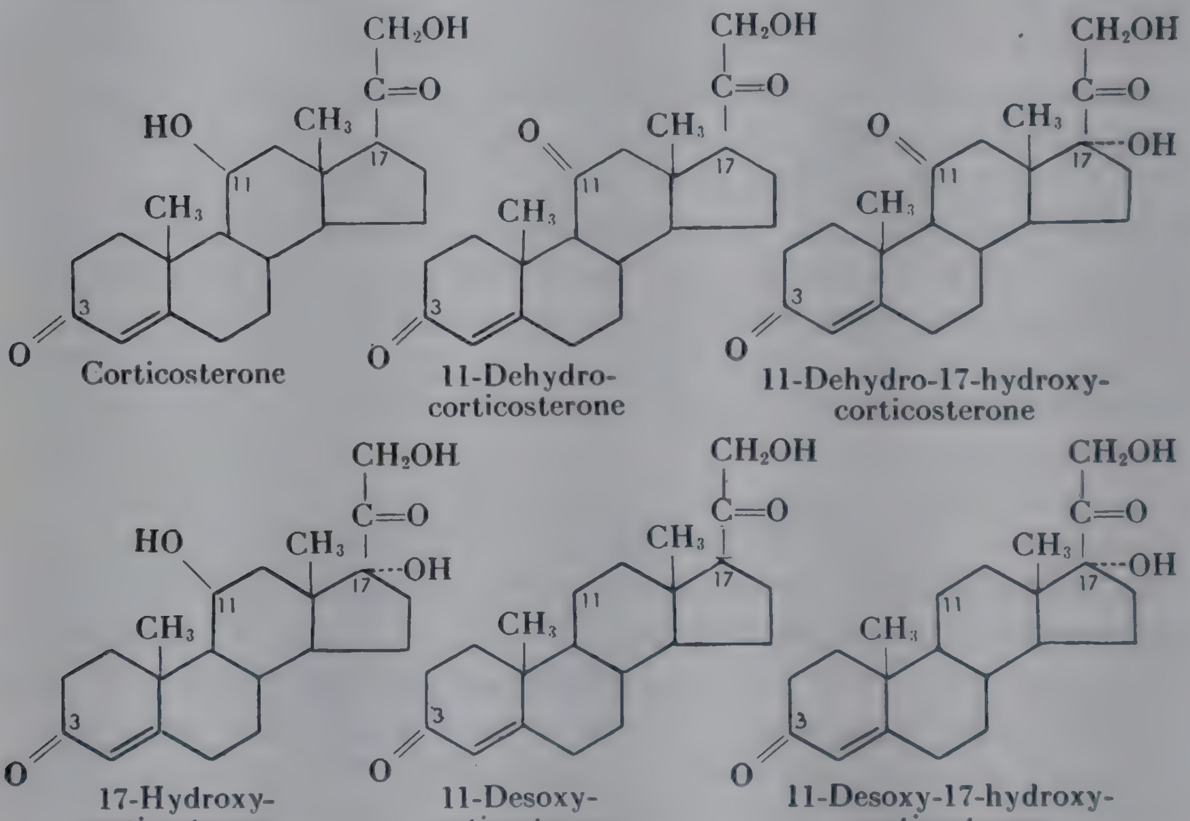
Certain additional structural features have a profound effect upon the biological activity of these compounds:

1. A hydroxyl (OH) group at C-21 enhances sodium retention and is necessary for activity in carbohydrate metabolism.

2. The presence of O (either as –OH or as =O, i.e., hydroxyl or ketonic groups) at C-11 is necessary for carbohydrate activity and decreases sodium retention.

3. A hydroxyl (OH) group at C-17 increases carbohydrate activity.

On the basis of these relationships between structure and biological activity, the adrenal cortical hormones may be classified in three categories: (1) Those possessing O at the C-11 position (either hydroxyl or ketonic), with activity in carbohydrate and protein metabolism and relatively less effect on electrolyte and water metabolism. These have been referred to as the "11-oxygenated corticosteroids" or "11-oxysteroids," and include corticosterone (Compound B), 11-dehydrocorticosterone (Compound A), 11-dehydro-17-hydroxycorticosterone (Compound E; cortisone), and 17-hydroxycorticosterone (Compound F; hydrocortisone); (2) Aldosterone (electrocortone), in which an oxygen at C-11 is linked also to a CHOH side-chain at C-18. This compound, now regarded as the major adrenal factor in the regulation of electrolyte metabolism, is much more active than desoxycorticosterone in this regard and in life-maintenance of adrenalectomized animals. It has an effect also in carbohydrate metabolism, about one-third that of cortisone; (3) Those without O at the 11-C position, with virtually no activity in carbohydrate or protein metabolism and profound effect on electrolyte and water metabolism. The most important representatives of this group are 11-desoxycorticosterone and 17-hydroxy-11-desoxycorticosterone (Compound S).



Corticosterone

11-Dehydro-corticosterone

11-Dehydro-17-hydroxy-corticosterone

17-Hydroxy-corticosterone

11-Desoxy-corticosterone

11-Desoxy-17-hydroxy-corticosterone

Fig. 44. Hormones of the adrenal cortex.

Androgenic 17-Ketosteroids. Three such compounds have been isolated from the adrenal cortex, all of which are 17-ketosteroids. The possibility has been suggested that their yield from adrenal tissues may be an artifact. However, androgenic compounds of 17-ketosteroid structure are found in the urine of normal individuals, and if these compounds are not actually produced by the adrenal cortex they are probably normal metabolites of adrenal products. In normal women, the adrenal cortex is probably the only source of androgens and neutral 17-ketosteroids; in men, about two-thirds are of adrenal origin and one-third are metabolites of testicular hormone. The latter information is derived from studies in castrates.

The chief 17-ketosteroid of normal, and of many abnormal urines is androsterone, whereas dehydroisoandrosterone is present under normal circumstances only in small amounts. The latter is present in large amounts in certain abnormal conditions (adrenocortical tumors). Androsterone is a representative of a group of 17-ketosteroids in which the hydroxyl group at C-3 is in the alpha position, dehydroisoandrosterone being a beta-17-ketosteroid. The alpha-17-ketosteroids can be separated from the beta compounds by digitonin precipitation, the beta-hydroxy compounds being precipitated, whereas the alpha-hydroxy compounds remain in solution. This separation of the total neutral 17-ketosteroids into alpha and beta fractions has been employed clinically (p. 420).

Dehydroisoandrosterone is a compound of considerable interest. It can be converted in the body into androsterone and etiocholanolone, and it has been suggested that it may be a precursor of these excretion products. Although it has not been isolated from the adrenal cortex, it may nevertheless be a primary secretion product of the latter. The increased urinary excretion of beta-17-ketosteroids, predominantly dehydroisoandrosterone, under pathologic conditions, would then be the result of incomplete metabolic conversion to androsterone and etiocholanolone.

On the whole, little definite information is available regarding adrenal precursors of urinary 17-ketosteroids. The adrenal cortex probably produces 17-ketosteroids, which may be the precursors of the urinary compounds. Corticosterone-like, 21-carbon-atom compounds may be metabolized into 17-ketosteroids.

The urinary 17-ketosteroids of normal individuals are partly androgenic, e.g., androsterone, isoandrosterone, and dehydroisoandrosterone, and partly biologically inactive, e.g., etiocholanolone. In cases of adrenal cortical tumor or hyperplasia the same compounds have been found, together with others. A number of related steroid compounds which lack the keto group at C-17 have also been isolated from urine. It is not yet definitely known whether the adrenal cortex, in pathologic states, produces other compounds or metabolizes normal compounds differently, or whether there is merely a quantitative shift, the "abnormal" compounds being present in normal individuals in such small amounts that they have escaped detection.

Estrogen. Estrone has been isolated from the adrenal cortex. It is probable that the small amounts of estrogens excreted by ovariectomized women originate in this organ. In rats spayed early in life the vagina opens later than in normal animals. (The vagina of the rat is closed at birth by a membrane which is later resorbed; the vagina opens under the influence of estrogens with the onset of puberty and the beginning of estrus cycling.) In adrenalectomized spayed rats, the vagina opens much later or not at all. In certain inbred strains of mice, early ovariectomy is followed by adrenal hyperplasia and tumors. Production of estrogen by these adrenal tumors is evidenced by the development of estrus changes in the vagina and by stimulation of growth of mammary cancer.

It is not certain whether the urinary estrogens of normal men are entirely of adrenal origin. They may be in part metabolites of testosterone, as was suggested for humans and as appears likely for the dog. Large amounts of estrogen can be extracted from the testes of the stallion, and in other species also the testis is probably producing some estrogen. However, production of estrogen by the adrenals plays an important role in certain pathological conditions (p. 436).

Progesterone. The adrenal cortex secretes progesterone or progesterone-like compounds. Desoxycorticosterone is convertible in the body to pregnanediol (p. 409), and dehydrocorticosterone to ketopregnanediol. Moreover, increased amounts of pregnanediol may be excreted by patients with Cushing's syndrome (p. 434).

Synthesis of Adrenal Hormones. Ever since the steroid nature of adrenal cortical and sex hormones was discovered and their structural relation to cholesterol was recognized, it was thought likely that cholesterol might be the precursor of steroid hormones.

Suggestive evidence was provided in the case of progesterone. After feeding cholesterol labeled with deuterium to pregnant women, pregnanediol, containing deuterium, was recovered from the urine. In the case of the adrenocortical steroids, older data suggest that cholesterol either is the mother substance or is in some way closely related to adrenal hormone formation. A single injection of adrenocorticotrophic hormone (p. 395) is promptly followed by a decrease in the cholesterol ester content of the adrenal cortex, which begins to revert to normal after twelve hours. Repeated injections of adrenocorticotrophic hormone do not decrease the cholesterol content of the cortex. This suggests that under the first impact of the stimulus the cholesterol present in the cortex may be rapidly utilized in the production of hormone, but if stimulation is continued the gland replenishes its cholesterol and deposits it at the same rate as it is being utilized for hormone production. More recently, it has been shown that the adrenal cortex is capable of synthesizing cortical steroids from both cholesterol and acetate. There are indications that cholesterol is not an obligatory precursor of steroid hormones in the biosynthesis from acetate, but rather that the synthesis proceeds to a common, hitherto unknown precursor, and then proceeds either to cholesterol or to the steroid hormone.

Perfusion of isolated adrenals has yielded important information. First, a number of steroids are secreted by the adrenal, chiefly Compound F (hydrocortisone), Compound B (corticosterone), and probably aldosterone. Furthermore, it has been shown that the adrenal cortex is capable of converting desoxycorticosterone to Compound F.

Ascorbic acid is present in the adrenal cortex in relatively large amounts. The ascorbic acid content generally follows a pattern similar to that followed by cholesterol; i.e., rapid decrease following a single injection of adrenocorticotrophic hormone, with subsequent return to normal values. The changes in ascorbic acid appear somewhat earlier than those in cholesterol. Whether and how ascorbic acid is involved in adrenal hormone production is not known. Decrease of adrenal ascorbic acid following injection of ACTH is now the accepted basis for assay of ACTH.

It appears that relatively small amounts of hormone are stored in the adrenal, most of it being passed rapidly into the blood stream. Under the influence of adrenocorticotrophic hormone, the blood levels of cortical hormone rise promptly and certain physiologic effects, such as lymphopenia and eosinopenia, occur within one-half hour, at a time when the depletion of cholesterol begins. This may indicate that synthesis and secretion of hormone occur almost immediately after the stimulus.

The "Alarm Reaction"; The Adrenal Cortex under Stress

A large number of stimuli, damaging agents, or conditions of unusual stress elicit in the body a typical series of events in which adrenal cortical hormone, or rather the pituitary-adrenocortical "axis," plays an important role. The nature of the alarming stimuli is totally nonspecific; they include almost every conceivable type of damage or change of usual environment, such as muscular exercise, exposure to cold, trauma, burns, drugs and poisons, bacterial infection, exposure to roentgen radiation, psychic influences, and many others. In response to these stimuli, a shock phase develops which, if not too severe and therefore lethal, is followed by a phase of "countershock." Continued exposure induces a period of resistance. The latter may break down and be followed by the stage of exhaustion. The shock phase will not be discussed here in detail. The mechanism of production of systemic manifestations of shock is still a matter of controversy. It is of interest that as an early response to alarming stimuli the output of epinephrine is increased. This is transient, producing a brief rise in blood pressure and blood sugar, followed by the hypotension and hypoglycemia of shock.

Adrenal cortical hormones appear to be necessary for recovery from the changes induced in the shock phase. Adrenalectomized (or hypophysectomized) animals exhibit a considerably decreased resistance to "stress" or alarming stimuli. Exposure to cold, readily tolerated by the intact animal, is fatal in the absence of the adrenals. Subcutaneous injection of relatively small amounts of histamine or formaldehyde kills the adrenalectomized animal, whereas it is tolerated by the intact animal. The intact animal responds to these nonspecific alarming stimuli with an increase in adrenal size. That this actually indicates increased function is suggested by the concomitant reduction in size of the thymus and other lymphatic structures, and by certain metabolic changes, such as negative nitrogen balance and tendency to hyperglycemia. A prompt increase in the level of cortical hormones (11-oxysteroids) in the blood can be demonstrated after application of an alarming stimulus. Resistance to many kinds of stress appears to be dependent upon adrenal cortical hormones, especially the 11-oxysteroids. The exact nature of their action in overcoming the shock phase of the alarm reaction is unknown.

The response of the adrenal cortex to an alarming stimulus (increased release and increased production of adrenal cortical hormone and eventual hyperplasia) is mediated by the adrenocorticotrophic hormone. In the hypophysectomized animal the adrenal cortex does not respond to such stimuli, which may consequently be fatal. The "alarming stimuli" cause the same changes in adrenal cholesterol and ascorbic acid as does injection of pure adrenocorticotrophic hormone.

The release of adrenocorticotrophic hormone from the anterior pituitary in response to various forms of stress is dependent upon hypothalamic centers located (in the dog) in the paramedian region of the anterior hypothalamus and at the junction of the middle and posterior hypothalamus. Experimental destruction of these nuclei prevents release of ACTH from the anterior pituitary following stress.

The following mechanism is suggested by recent experiments. Various impulses resulting from stress reach the hypothalamus through nervous pathways. In the hypothalamic nuclei a hormonal factor is liberated (secreted), which in turn fires the anterior pituitary to release ACTH. Since stalk section does not abolish ACTH release, it is suggested that the neurohumoral factor reaches the anterior pituitary through the systemic circulation. Inasmuch as injection of epinephrine in intact animals and humans causes release of ACTH from the pituitary, and since epinephrine release from the adrenal medulla has long been known to occur under various stresses (p. 421), it was thought that epinephrine was a necessary mediating agent, the initial stimulus causing releases of epinephrine, the latter setting the subsequent steps of the alarm reaction in motion. It would now appear that epinephrine release is not an indispensable factor, but it probably plays a role in the early stages of the alarm reaction. Another factor suggested as important in the mechanism of ACTH release is the level of cortical hormone in the tissues. The assumption is that the stressing, damaging agent first causes a decrease of cortical hormone in the body fluids (using it up?), and that the lowered level of cortical hormone sets off the mechanism of release. Whereas such a decrease in hormone level under stress has not been demonstrated, it has been found that ACTH release does not take place if the cortical hormone levels are raised (by injecting adrenal cortical extract or cortisone) prior to the stress.

The two suggested mechanisms of ACTH release are not mutually exclusive; a dual mechanism, involving an "autonomic phase" (by way of epinephrine) and a "metabolic phase" (mediated through variations of blood levels of cortical hormones) has been proposed.

11-Oxysteroids, and perhaps 11-desoxysteroids or aldosterone, but not 17-ketosteroids (androgens), are probably produced in excess for some time following an alarming stimulus. In humans, in the early stages of the alarm reaction there is increased excretion of both 11-oxysteroids and 17-ketosteroids, but the latter rapidly fall off whereas the increased excretion of the former continues. Since the oxysteroids are protein catabolizers, they induce negative nitrogen balance.

In animals (rats and dogs), prolonged administration of large amounts of desoxycorticosterone and sodium chloride has produced vascular and renal changes (nephrosclerosis) and changes in the joints. It has been hypothesized that, in man, nephrosclerosis, hypertension, certain diseases of the arteries (sclerosis, periarteritis nodosa), diabetes, and some arthritides might be the aftermath of repeated successful adaptations to alarming stimuli, with prolonged oversecretion of adrenal cortical hormones ("diseases of adaptation"). However, evidence is accumulating that large amounts of adrenocortical hormone act, at least quantitatively, differently under conditions of stress than under homeostatic conditions. It would appear that some of the damaging effects of large amounts of cortical hormone occur only in the homeostatic condition, in the absence of "need" for such amounts. Under stress there is such "need" and the cortical hormone is "used" to restore homeostasis without causing damage. The quotes indicate that the terms are used in a descriptive sense only; we know nothing about the mechanisms involved. However, these observations and concepts will have to be considered in evaluating the theory of diseases of adaptation.

It has been shown that mental and emotional stress may act as an "alarming stimulus," causing an increased release of adrenocortical hormone. Thus, for example, individuals working with the "pursuit meter," under considerable mental strain, but comfortably seated in a chair, showed increased excretion of 17-ketosteroids. Whether the mediating factor in a psychologically induced "alarm reaction" is increased liberation of epinephrine is not yet certain. These observations constitute an important link between the psychological and somatic aspects of many "psychosomatic" phenomena.

Methods of Assay. These may be divided into two categories, (*a*) biological and (*b*) chemical.

(*a*) Bioassay Procedures. These are based on certain biological actions of the adrenocortical hormones in adrenalectomized animals: (1) maintenance of life; (2) improvement of work performance; (3) decrease in urinary excretion of sodium; (4) prolongation of survival during exposure to cold; (5) promotion of glycogen deposition in the liver. Of these, the last two (cold protection and liver glycogen deposition) have been used successfully for quantitative purposes. They measure only those adrenal steroids oxygenated at C-11 (11-oxysteroids), and appear to be specific for these substances. However, these procedures are time-consuming and expensive, and have been largely supplanted, for routine purposes, by less specific chemical methods.

(*b*) Chemical Procedures. All of the known biologically active adrenal steroids contain an α-ketol side chain at C-17 (Ring D) and an α,β-unsaturated 3-ketone grouping (Ring A) (Fig. 44, p. 424). These structural features constitute the basis for the following chemical assay procedures (colorimetric).

(1) *Formaldehydogenic Method.* The α-ketol or glycol side-chains of steroids react with periodic acid (glycol cleavage) with generation of formaldehyde, which may be determined quantitatively.

(2) *Phosphomolybdic Acid Reduction.* Steroids containing a primary or secondary (but not tertiary) α-ketol grouping, or an α,β-unsaturated 3-ketone grouping, can reduce phosphomolybdic acid (e.g., corticosterone, desoxycorticosterone, progesterone, etc.).

(3) *Copper Reduction.* Copper is reduced by steroids containing an α-ketol grouping. An α,β-unsaturated 3-ketone grouping does not reduce copper; consequently, compounds such as progesterone do not interfere with this procedure.

(4) *Porter-Silber Reaction.* The 17-hydroxy types of corticoids (e.g., Compound F) produce a yellow color with phenylhydrazine and sulfuric acid. Various modifications of this reaction are perhaps the most widely used corticoid assay procedures at the present time.

These chemical reactions are not specific for cortical hormones, being given by other normal constituents of body fluids. However, a rather satisfactory degree of specificity is attained by the extraction procedures employed preliminary to application of the chemical assay reactions. Chemical methods yield consistently higher values (in urine) than do biological methods. On the other hand, the desoxycorticosterone type of compound will give certain of the chemical but not the usual biological reactions (cold protection and liver glycogen deposition). The formaldehydogenic and Porter-Silber reactions yield values which correlate more consistently with biossay values than do those obtained with reduction tests.

Metabolism and Excretion. On the basis of assays of adrenal vein blood, it appears that the adrenals produce relatively large amounts of hormones, which are released rapidly into the circulation. The compound secreted in largest quantity is 17-hydroxycorticosterone (compound F). The adrenocortical hormones apparently undergo rapid changes in the organism, with loss of biological activity. *In vitro* studies indicate that both the 11-oxygenated and the 11-desoxy types of hormone are inactivated by liver, and also perhaps by other tissues. The exact nature of these changes or of the end products is not known. A portion of the corticoids also undergoes conjugation in the liver, forming glucuronides. Only a small fraction, probably about 5 to 10 per cent, of the quantity of hormone produced is excreted in the urine in biologically active form.

Employing the Porter-Silber reaction, the blood plasma of normal adult subjects has been found to contain about 2 to 34 (av. 13) μg. 17-hydroxycorticoids per 100 cc. A large fraction of the plasma corticoids is in conjugated form (glucuronide).

Information regarding the metabolism of adrenocortical hormones has been obtained by studying changes in urinary steroids after (1) adrenalectomy (or in Addison's disease), (2) administration of ACTH and (3) of cortical hormones (or in adrenal hyperfunction). The following compounds or classes of compounds have been identified as being presumably of adrenal cortical origin:

NEUTRAL 17-KETOSTEROIDS. These substances, determined colorimetrically (Zimmermann reaction), give a measure of metabolites of adrenal and testicular hormones (p. 416) excreted as 17-ketones. In normal adult subjects, the adrenal is responsible for about two-thirds of the total urinary neutral 17-ketosteroids in men (total, 7–20 mg. daily) and for the total quantity in women (5–15 mg. daily). They can result from the catabolism of adrenal corticoids and from secretion of adrenal androgens (p. 425).

BIOLOGICALLY ACTIVE CORTICOSTEROIDS. The urine contains steroids which exert biological effects characteristic of adrenal cortical hormones. Two have been identified in normal subjects: 17-hydroxycorticosterone (Compound F); 11-dehydro-17-hydroxycorticosterone (Compound E). These probably represent an overflow from the circulation, the quantity excreted daily being 0.15 to 0.4 mg. in normal adult men and 0.1 to 0.3 mg. in women (mg. equivalents of 11-dehydrocorticosterone, assayed by liver glycogen deposition method).

NEUTRAL FORMALDEHYDOGENIC STEROIDS. Substances in this category are excreted normally in quantities of 0.8 to 2.5 mg. daily (mg. equivalents of 11-desoxycorticosterone), the values for men being somewhat higher than for women.

PORTER-SILBER REACTING STEROIDS. The urine of normal adults contains steroids (17-hydroxycorticoids) that give this reaction, the quantity excreted daily ranging from 0.8 to 10 mg. (after hydrolysis with glucuronidase). It should be pointed out that chemical methods for assay of corticoids are currently in a state of flux. None of the available procedures is entirely satisfactory, and normal values reported from different laboratories vary rather widely.

IDENTIFIED METABOLITES. A number of adrenal hormones and their metabolites have been isolated from the urine of normal subjects. In addition to the two carbohydrate-active compounds indicated above, these include pregnanediol (0.1 mg. daily) and three 17-ketosteroids (dehydroisoandrosterone, 11-hydroxyandrosterone, and etiocholanolone). Pregnanediol is probably derived from desoxycorticosterone as well as from progesterone, which is produced by both the adrenal cortex and the corpus luteum of the ovary. Aldosterone is probably the precursor of the desoxycorticosterones, and is believed to be the primary and major "salt-active" hormone formed by the adrenal cortex.

Metabolic Effects. Absence of adrenal cortical hormones (adrenalectomy; Addison's disease) or administration of excessive amounts of these hormones are accompanied by characteristic metabolic changes, which may be grouped broadly under two headings: (1) electrolyte and water metabolism; (2) carbohydrate, protein, and fat metabolism. The latter category is influenced virtually solely by the 11-oxygenated cortical hormones, whereas the former is influenced predominantly by aldosterone and the 11-desoxy type of hormone, but also by 11-oxysteroids.

ELECTROLYTES AND WATER. Adrenalectomized animals and patients with Addison's disease, if untreated, exhibit the following phenomena: (1) increased urine volume, with (2) disproportionately increased excretion of Na and Cl, leading to (3) decreased concentration of Na and Cl in blood plasma, (4) decrease in body water (dehydration) and (5) decrease in plasma volume. Ultimately there is a fall in blood pressure, with circulatory collapse and impaired renal glomerular filtration, which are aggravated by, but not necessarily dependent upon, the changes in electrolyte and water balance. Simultaneously with the changes in Na, the urinary excretion of K decreases and the concentration of K in the plasma increases.

The kidney is apparently the organ primarily involved in these changes in Na, and perhaps also in K metabolism. In the absence of adrenal hormones, the renal tubular epithelial cells appear to be unable to reabsorb Na (also Cl and water) adequately from the glomerular filtrate in spite of a low plasma Na concentration. They also are apparently unable to excrete K adequately (or they reabsorb it excessively) despite the increased plasma K concentration. At the same time there is a disturbance in the equilibrium between the extracellular (plasma and interstitial fluids) and intracellular fluid compartments, characterized by an increase in intracellular (muscle) water and K and a decrease in Na and Cl.

These abnormalities can be prevented or corrected by administration of desoxycorticosterone or the 11-oxygenated adrenal steroids (e.g., corticosterone, Compounds E and F), the Na-retaining potency of the latter being about 25 to 50 per cent that of the former. There are also qualitative differences in the actions in this connection of these two types of hormone. In the absence of the adrenals, Na is not excreted adequately when unusually large quantities are given. This difficulty is aggravated by desoxycorticosterone, which further increases retention of Na, but is corrected by adrenal cortical extracts or Compound E. Moreover, the elevation of plasma Na resulting from excessive doses of desoxycorticosterone in intact animals can be prevented by simultaneous administration of cortical extract or ACTH. It would appear that desoxycorticosterone acts unidirectionally, inducing Na retention and K excretion under all circumstances, whereas the 11-oxygenated adrenal hormones maintain homeostasis in this sphere, tending to preserve and restore the Na equilibrium regardless of the direction of deviation from the normal. As indicated elsewhere (p. 424), the major "salt-active" hormone elaborated by the adrenal cortex is probably aldosterone, which is enormously more active in this regard than is 11-desoxycorticosterone.

Carbohydrate Metabolism. The effects of adrenal cortical hormones on carbohydrate metabolism are discussed in detail elsewhere (p. 16). These actions are exhibited by only the 11-oxygenated corticoids, which (1) decrease utilization of glucose, and (2) increase gluconeogenesis from protein. Consequently, excessive amounts of these hormones may, in certain species, induce a diabetic state (e.g., Cushing's syndrome), while adrenal insufficiency (Addison's disease) is characterized by a tendency toward hypoglycemia and by abnormal sensitivity to insulin.

Protein Metabolism. Administration of relatively large amounts of ACTH, adrenal extracts, or 11-oxygenated cortical hormones to fasting intact animals is followed by an increase in urinary nitrogen and a negative nitrogen balance (protein catabolic effect). As indicated above, this is accompanied by an increase in body carbohydrate, and G : N ratios indicate that about 50 to 70 per cent of the extra protein catabolized is converted to glucose. Exogenous protein is apparently not affected in this manner.

There is evidence that more physiological amounts of cortical hormone, on the contrary, exert a protein anabolic effect. Administration of ACTH increases the positive nitrogen balance in children, and cortical hormones, in certain dosages, reduce nitrogen excretion in the urine. It would appear that, as is true of other hormones (e.g., thyroid), the quantity secreted and the functional status of the target cells have an important bearing on the character of the metabolic response.

The mechanism of action of these cortical hormones in protein metabolism is not entirely clear. There is some evidence that they increase deamination of amino acids and that adrenalectomized fasted animals do not convert deaminized amino acid residues to glucose as readily as do normal animals. However, protein-fed adrenalectomized animals apparently behave normally in this respect. It is difficult to reconcile these observations. The suggestion has been made that the 11-oxygenated adrenal hormones may exert an "antianabolic" rather than, or in addition to a catabolic action on protein.

LIPID METABOLISM. Adrenocortical hormones (11-oxygenated) (1) depress synthesis of fat from carbohydrate, (2) increase mobilization of depot fat to the liver, and (3) increase ketogenesis in the liver. Conversely, adrenalectomy prevents the excessive mobilization of depot fat that follows fasting, stress, or administration of anterior pituitary extracts (growth hormone). It also decreases the ketosis that occurs in fasting, fat-fed, or phlorizinized or depancreatized animals.

Although these adrenal hormones are necessary for the mobilization of depot fat and excessive ketogenesis under the circumstances mentioned, they are incapable of producing these effects in the absence of an anterior pituitary factor ("fat-mobilizing hormone"), which may be the growth hormone. Simultaneous action of both is required, the latter acting as a trigger mechanism in the presence of adequate amounts of cortical hormone. Both factors also apparently partially inhibit lipogenesis from carbohydrate, adrenalectomized animals tending to utilize a larger proportion of glucose over pathways of oxidation and fat synthesis. In this respect, too, the action of the adrenal hormones is antagonistic to that of insulin, which increases lipogenesis from carbohydrate.

Adrenocortical Hyperfunction

From the standpoint of hormone secretion, recognized syndromes due to adrenocortical hyperfunction may be classified as follows:

1. Increased secretion of all hormone groups: Cushing's syndrome (panhypercorticalism).
2. Exclusive hypersecretion of androgens: adrenogenital syndrome.
3. Exclusive hypersecretion of estrogens: feminizing syndrome (in males).
4. Exclusive hypersecretion of 11-oxysteroids: diabetes of adrenal origin.

There may be instances in which there is overlapping of two or more of these categories. In certain cases of the adrenogenital syndrome, for example, the adrenals, instead of exclusively oversecreting androgens, may "almost exclusively" oversecrete them, producing also one or more of the other hormones somewhat in excess. The latter may give rise to symptoms, thereby causing pictures intermediate between the typical syndromes.

Cushing's Syndrome. This condition is associated usually with hypersecretion of all types of adrenocortical hormones (adrenal tumor or hyperplasia), although not necessarily at all stages of the disease. There is usually also an excess of basophiles in the anterior pituitary (basophilic adenoma).

CORTICOIDS. Excessive amounts of 11-oxysteroids are excreted in the urine rather consistently. Increased concentrations have been found in the blood. Differentiation between cases due to adrenal hyperplasia and those due to adrenal tumor may be aided by observing the effects of administration of ACTH or cortisone on the urinary corticoid excretion.

In normal subjects and usually in patients with adrenal hyperplasia, the urinary corticoids increase after administration of ACTH and decrease after therapeutic doses of cortisone. In many cases of hyperfunctioning adrenal tumor with Cushing's syndrome, the urinary corticoid excretion is not influenced significantly by such treatment, the tumor usually not being under hormonal control.

17-KETOSTEROIDS. Urinary 17-ketosteroids may be within normal limits, but are usually moderately increased (20–30 mg./24 hrs.). Very high values are observed occasionally. As indicated elsewhere (p. 420), fractionation into alpha and beta fractions may at times be helpful in distinguishing between increase due to adrenal hyperfunction and that due to other causes (p. 420), as well as between adrenal hyperplasia and adrenal tumor. However, this procedure is often not helpful in differential diagnosis.

Whereas administration of cortisone is followed in normal subjects by a decrease in urinary 17-ketosteroids, this often does not occur in Cushing's syndrome. However, administration of ACTH usually produces an increase (often excessive), as it does in normal subjects.

SEX HORMONES. High urinary pregnanediol values have been reported in several cases. Since these women were amenorrheic, as is common in Cushing's syndrome, this high excretion may be the result of excessive production of desoxycorticosterones (p. 426).

Urinary estrogen excretion is usually low, but high values have been reported occasionally.

CARBOHYDRATE METABOLISM. Diabetes mellitus is a very constant feature of Cushing's syndrome, although not present in all stages. The development of frank diabetes is preceded by a stage of latent or subclinical diabetes, evidenced by diminished glucose tolerance. The diabetes is evidently attributable to the increased secretion of 11-oxysteroids, which increase gluconeogenesis and dedecrease carbohydrate utilization, thereby leading to hyperglycemia and to secondary changes in the islets of Langerhans (p. 70).

PROTEIN METABOLISM. Negative nitrogen balance occurs as a result of excessive protein catabolism due to the action of oxysteroids. A number of manifestations, including demineralization, thinning of the skin, purplish striae, and, in part, diabetes, have been attributed to increased protein catabolism. It is of interest that protein catabolism is increased in Cushing's syndrome in spite of the simultaneous hypersecretion of protein anabolizing androgens. The latter are evidently not increased sufficiently to overcome the protein catabolic action of the oxysteroids. The fact that therapeutic administration of additional androgens may result in a positive nitrogen balance suggests that the matter is merely one of relative amounts of the two antagonistic hormone groups secreted by the adrenal cortex.

Na, Cl, AND K METABOLISM. The characteristic changes in Cushing's syndrome are: decreased urinary excretion of Na and Cl, and increased excretion of K. Excessive loss of K is accompanied by development of a state of hypochloremic alkalosis (p. 342).

The electrolyte changes are probably not the result of excess production of aldosterone or desoxycorticosterone alone. 11-Oxysteroids, especially Compounds F and E, influence sodium, potassium, and chloride metabolism. It would seem that, if present in large amounts, they may cause changes similar to those caused by desoxycorticosterone, although they are much less potent in this respect than the latter. It is therefore difficult to ascribe the electrolyte changes in Cushing's syndrome entirely to one or the other group of compounds, unless there is other evidence of excessive production of desoxycorticosterone, e.g., increased pregnanediol excretion (p. 434). Under certain experimental conditions, changes suggestive of an antagonism between 11-desoxycorticosterone and 11-oxysteroids have been observed. A single injection of 11-desoxycorticosterone causes sodium and chloride retention in normal subjects, but induces sodium and chloride loss in cases of Cushing's syndrome.

CALCIUM, PHOSPHORUS, AND PHOSPHATASE. The osteoporosis so frequently present has prompted extensive studies of calcium and phosphorus metabolism. These electrolytes are present in the serum in normal amounts. However, a negative calcium balance has been demonstrated; this loss of calcium may be due to the inability of the bony matrix to take up calcium salts. The serum alkaline phosphatase is usually slightly elevated. This may be interpreted as due to increased osteoblastic activity, i.e., a futile attempt on the part of the bones to compensate for an inadequacy of the matrix by overactivity of the cells.

EOSINOPENIA. The average normal eosinophil count is 100 to 300 per cu. mm. blood. Administration of 11-oxysteroids or ACTH to normal subjects usually results in a prompt drop of at least 50 per cent of the pre-injection value. In patients with Cushing's syndrome, the basal eosinophil count is often below 50 per cu. mm., a consequence of the increase in circulating 11-oxysteroids.

Adrenogenital Syndrome. This designation is applied to cases of adrenocortical hyperfunction in women, manifested by virilization and no other metabolic changes. It may occur in young boys, in whom it is characterized by precocious development (skeletal, sexual).

The urinary 17-ketosteroid excretion is typically high (adrenal androgens); the urinary corticoids are normal. This is at times helpful in distinguishing between virilization due to adrenal hyperfunction and that due to certain ovarian tumors. In the latter, the urinary 17-ketosteroids may be normal or only moderately increased. ACTH has been reported to be increased in the blood of certain children with the adrenogenital syndrome.

Feminizing Syndrome in Men. This is a rare condition associated with carcinoma of the adrenal cortex. There are very few reported studies of estrogen excretion; the recorded values are high. It may be assumed that this should be a constant finding.

The urinary 17-ketosteroids may be increased also in some cases of feminizing, estrogen-producing adrenal tumors.

Miscellaneous Conditions. Whereas diabetes occurs commonly in Cushing's syndrome, and is absent in the adrenogenital syndrome, an instance has been reported of an adrenortical tumor manifested by diabetes, with no other evidence of adrenocortical hyperfunction. This is of theoretical importance because of the implication that diabetes mellitus may be due in some instances to adrenocortical hyperfunction limited to secretion of 11-oxysteroids.

Transitory states of hyperfunction occur under a variety of circumstances in which the organism is subjected to unusual stress (alarm reaction, p. 427). These include: trauma, burns, surgical operations (immediate postoperative state), exposure to cold, psychic influences, unusual emotional stress, pain, infection, etc. Under such circumstances, the urinary excretion of corticoids and 17-ketosteroids increases, and eosinopenia may occur (p. 429).

Adrenocortical Hypofunction

Adrenocortical hypofunction may be caused by a destructive primary lesion of the adrenal glands (Addison's disease). It may also be secondary to hypofunction of the anterior pituitary, in which case lack of adrenocorticotrophic stimulation causes atrophy of the adrenal cortex with depression of function. Furthermore, adrenocortical insufficiency may be caused by overwhelming infections, in which destruction of the cortex is preceded by an excessive "demand" for cortical hormone in response to the "stress" of the infection. There is suggestive evidence that excessive functional stimulation may actually cause not only functional exhaustion, but also cell damage and necrosis.

Corticoids. Urinary 17-ketosteroids are usually very low or absent in women with Addison's disease (less than 1 mg. per 24 hours). In men, the values are somewhat higher (3–4 mg. per 24 hours) because of androgen production by the testes. Similar values may be encountered in panhypopituitarism (secondary hypoadrenocorticalism).

Although very useful, especially in women, determination of 17-ketosteroids is not a specific diagnostic procedure in Addison's disease. As indicated elsewhere (p. 418), low values may be obtained also in myxedema, undernutrition, hepatic disease, nephritis, anorexia nervosa, and other debilitating diseases.

Carbohydrate Metabolism. The lack of oxysteroid secretion results in impairment of carbohydrate metabolism (p. 16). The most important defects in this connection are (1) impairment or cessation of gluconeogenesis, and (2) increased utilization of glucose by the tissues. In addition, there is impairment of absorption of carbohydrate from the intestine, although it is not certain that this is due specifically to the lack of oxysteroids.

Clinically, the result of these deficiencies is a tendency toward fasting hypoglycemia. There is considerable variation in the extent of this disturbance in different patients. Fasting blood sugar levels (after the conventional overnight fast) tend to be on the low side of normal, or slightly subnormal, but some patients exhaust their carbohydrate stores so rapidly and so completely that even shorter periods of fasting may lead to severe hypoglycemic manifestations. Any condition which further increases carbohydrate utilization will increase the tendency to spontaneous hypoglycemia. Such conditions include severe infectious diseases or exercise.

Oral glucose tolerance tests are characterized by an inadequate rise of blood sugar ("flat curve"), due chiefly to inadequate absorption. However, this type of curve is encountered so frequently in other conditions that it is of practically no diagnostic value (p. 52).

Intravenous Glucose Tolerance Test (p. 51). Following intravenous infusion of glucose, the blood sugar curve is normal during the first phase, returning to the fasting level in about two hours. At about the third hour, the blood sugar, instead of leveling off after a slight "dip," may continue to fall (three to seven hours) to severely hypoglycemic levels (prolongation of hypoglycemic phase). This disturbance of carbohydrate metabolism, resulting from absence or inadequate secretion of "sugar hormone" (11-oxysteroids), is demonstrable in about 60 per cent of patients with Addison's disease. A normal response to glucose therefore does not rule out Addison's disease. Nor is the abnormal hypoglycemic response indicated above obtained exclusively in this condition. A similar response may be observed in panhypopituitarism (p. 51) and in hyperinsulinism (p. 48).

Insulin Tolerance Test (Hypoglycemia Irresponsiveness, p. 53). Following intravenous injection of a standard dose of insulin (0.1 or 0.05 units/kg.) in the fasting state, the blood sugar normally falls rapidly, attaining minimum levels of about 30 to 40 mg. per cent in twenty to thirty minutes and returning to the fasting level in one and a half to two hours. In patients with Addison's disease, the blood sugar usually falls to 20 mg. per cent or less, and remains at hypoglycemic levels for prolonged periods, sometimes showing no indication of an attempt to rise from the minimum levels attained. In most cases, severe manifestations of hypoglycemia are induced, including coma, and the test procedure must be interrupted by intravenous injection of glucose. As in the case of glucose-induced hypoglycemia, this phenomenon is due to a lack of adrenal cortical hormones to counteract the effect of the added insulin.

An abnormal response to insulin is found in almost all patients with Addison's disease, including the great majority of those who fail to exhibit severe hypoglycemia with the intravenous glucose tolerance test. This may be due to the fact that more insulin may be administered than is produced endogenously in response to glucose, and that the exogenous insulin is given rapidly at one time. Furthermore, the fasted patient with Addison's disease is probably near the point of exhaustion of the hepatic glycogen reserves, which may be partially replenished by administration of glucose.

"Hypoglycemia irresponsiveness," induced by insulin, is not diagnostic of Addison's disease; it is found also in panhypopituitarism, resulting in this instance, in part at least, from hypofunction of the adrenal secondary to lack of adrenocorticotrophin. It may occur also in starvation, probably due chiefly to depletion of liver glycogen.

Because injection of insulin in fasted patients with Addison's disease is usually followed by hypoglycemic coma, this test should be carried out with the utmost care, having glucose solution and sterile syringes at hand to cope with the emergency. Because of its inherent danger, this procedure is not recommended as a routine test. A modified procedure has been suggested, regular insulin being given before meals in increasing doses. Increased insulin sensitivity is manifested by a tendency to hypoglycemia under these conditions, and the danger of inducing coma is small. It has been suggested that the diagnosis of Addison's disease can be excluded if the subject tolerates 10 units of regular insulin three times daily without developing symptoms of hypoglycemia.

Another method of demonstrating hypoglycemia irresponsiveness with a greater degree of safety is the insulin-glucose test (p. 31). The patient receives 0.1 unit of crystalline insulin per kilogram body weight intravenously, and thirty minutes later, or at the onset of hypoglycemic manifestations (whichever comes first), 0.8 gm. glucose per kilogram body weight by mouth. Normally the glucose causes a marked rise in blood sugar, reaching a peak within sixty minutes. In cases of Addison's disease and of panhypopituitarism the rise in blood sugar is significantly decreased and delayed. Inasmuch as the orally administered glucose is occasionally vomited, it is wise to have solutions of glucose at hand for intravenous administration to combat hypoglycemic manifestations that may occur as a result of the insulin.

Electrolyte and Water Metabolism. Deficient secretion of corticoids results in loss of sodium, chloride and water, and retention of potassium. The lack of secretion of Compound F and cortisone, the action of which on electrolytes is qualitatively similar although quantitatively much weaker than that of aldosterone or desoxycorticosterone, contributes to the electrolyte disturbance. The loss of sodium is somewhat in excess of that of chloride. As a result of this electrolyte disturbance there is a tendency toward hemoconcentration, dehydration, and acidosis; the tendency of the pattern of serum electrolytes is toward low chloride and sodium and elevated potassium values.

However, in chronic stages these changes are often not sufficiently marked to be of diagnostic value (p. 285). Only extreme changes are recognizable by study of the concentrations of serum electrolytes. These changes are characteristically present in Addisonian crisis, i.e., hypochloremia, low serum sodium, elevated serum potassium, acidosis, and hemoconcentration. It is understandable that many factors precipitating crisis in the Addisonian patient may operate by aggravating the electrolyte imbalance present in the chronic stage, e.g., profuse sweating, diarrhea, pneumonia, etc.

In the chronic stage, provocative diagnostic procedures must be employed to make the underlying disturbance of electrolyte and water metabolism manifest. One group of such test procedures is based on the fact that the normal individual on a restricted sodium chloride intake will conserve sodium and chloride by adequate reabsorption in the kidney tubules. Any excess of potassium is promptly eliminated in the normal individual. This renal control of electrolyte balance is impaired in the absence of cortical hormones.

Another procedure is based on the fact that the patient with Addison's disease cannot respond with prompt diuresis to the ingestion of water. Desoxycorticosterone appears to have a diuretic effect, diminishing reabsorption of water by the kidney tubules; under many circumstances this effect is masked by the water retention incident to the sodium and chloride retention caused by desoxycorticosterone. The water test, indicated below, is based on lack of this diuretic response.

SALT DEPRIVATION TEST (CUTLER-POWER-WILDER). The patient receives a special salt-poor diet for two and one-half days. This contains 0.95 gm. Cl, 0.59 gm. Na, and 4.1 gm. K daily. Distilled water is given as drinking water during this period. In addition, the following measures are carried out.

First day: Free fluid intake is encouraged, but not measured. In the afternoon, the patient is given potassium citrate, 93 mg. per kg. body weight.

Second day: The patient receives measured amounts of fluids, totalling 40 ml. per kg. body weight. Potassium citrate is given as on the previous day.

Third day: Fluid intake is 20 ml. per kg. This must be taken before 11:00 A.M. Urine is collected from 8:00 A.M. to 12:00 noon, and analyzed for chloride concentration and, if possible, for sodium concentration.

In normal individuals, and in most diseases not involving the adrenal cortex, the chloride concentration is less than 125 mg. per 100 cc. urine, and the sodium concentration is less than 100 mg. per 100 cc. urine.

Concentration of chloride of 250 mg. per 100 cc., and of sodium of 200 mg. per cc., or more, are encountered almost only in adrenal insufficiency (Addison's disease; adrenal cortical failure secondary to panhypopituitarism).

This test is designed to intensify the most important metabolic defect of Addison's disease, the salt hormone deficiency. Consequently, the clinical condition usually deteriorates during the two and one-half day test period, the blood pressure declines, weight is lost, and hemoconcentration may progress (rising hematocrit values). The deterioration of the subjective and objective manifestations of the disease does not always occur; its absence does not rule out Addison's disease if the sodium and chloride excretion is significantly high.

WATER TEST (ROBINSON-KEPLER-POWER).

Procedure I. No fluid is permitted after 6:00 P.M. on the evening preceding the test. At 10:00 P.M. the patient voids; this specimen is discarded. Thereafter all urine is collected until 7:00 A.M., when the patient voids again; this is included in the night specimen. Immediately thereafter, the patient drinks, as rapidly as possible, 20 cc. water per kg. body weight. Urine specimens are obtained at 8:00 A.M., 9:00 A.M., 10:00 A.M., and 11:00 A.M. The volume of each urine specimen is recorded.

Normally, the volume of one or more of the day specimens exceeds that of the night specimen. In Addison's disease, water elimination is delayed, and consequently the volume of the night specimen is greater than any one of the day specimens. The test is not specific, because factors other than adrenocortical insufficiency may cause delayed elimination of water, such as cirrhosis of the liver, nephritis, and cardiac failure. A normal response is believed to be strong evidence against Addison's disease. An abnormal response calls for further study.

Procedure II. This can be carried out simultaneously with Procedure I. At 7:00 A.M., before the ingestion of water, a blood sample is drawn; plasma chloride and urea nitrogen are determined. Chloride and urea nitrogen are determined in the night urine specimen.

An empirical combined chloride-urea-and-water clearance formula is employed as follows:

$$A = \frac{\text{Urine urea N (mg.\%) (night spec.)}}{\text{Plasma urea N (mg.\%)}} \times \frac{\text{Plasma Cl (mEq./L.) (night spec.)}}{\text{Urine Cl (mEq./L.) (night spec.)}} \times \frac{\text{Vol. largest urine day spec. (cc.)}}{\text{Vol. night urine spec. (cc.)}}.$$

The value for A is usually above 30. Values of less than 25 are strongly suggestive of Addison's disease, if nephritis can be ruled out.

This test should be carried out only in a hospital. Nursing staff and house officers must be aware of the potential dangers. The patient must be carefully observed, with blood pressure readings every three hours. If necessary, the test must be interrupted and glucose-saline and adrenal cortical hormones administered intravenously for impending crisis.

The physician should ascertain personally, before the test is started, that everything needed for the emergency treatment (infusion set-up, sterile glucose-saline solution, adrenal cortical hormones) is readily available.

Because of these dangers, this test procedure has been largely replaced by the "water test" outlined below. However, both have been generally supplanted by studies of hormone excretion and responses to administration of ACTH.

Miscellaneous Abnormalities. The BMR is usually within normal limits, but low values are observed occasionally. Weight loss may be a contributory factor, as in the case of starvation (p. 376). Creatinuria occurs regularly in untreated cases. It is known to occur also in many conditions accompanied by muscle wasting (p. 181).

Kidney function is seriously impaired in crisis, apparently due largely to the low blood pressure (glomerular filtration pressure inadequate), but also in part to the abnormal electrolyte concentrations. When the blood pressure drops below 50 mm. Hg, urine formation ceases entirely. Even before complete anuria occurs, there is a rise in NPN and blood urea N, and a decrease in urea clearance; PSP excretion is reduced.

In periods between crises, with the blood pressure and circulation fairly adequately maintained, kidney function may be normal. However, the urea clearance and concentration tests may reveal impairment of renal function in some cases, due presumably to some effect of the electrolyte imbalance.

As has been mentioned elsewhere (p. 432), the site of action of the salt metabolism hormone is probably the kidney tubule. This has been clearly shown in adrenalectomized animals, but findings in patients with Addison's disease have been somewhat equivocal. It has been found, for example, that the decrease in glomerular filtration is greater than can be accounted for on the basis of diminished blood flow.

Special Diagnostic Procedures. Diagnosis of adrenocortical insufficiency and, specifically, of Addison's disease, has been facilitated by the introduction of certain procedures which test the capacity of the adrenals to respond to stimulating doses of ACTH.

Four-Hour ACTH Test. An eosinophil count is made and 25 mg. ACTH are injected intramuscularly. A second eosinophil count is made four hours later. Because of rather wide spontaneous fluctuations in eosinophils during the morning hours, this procedure should be conducted in the afternoon.

In normal individuals, the eosinophils decrease 50 per cent or more 4 hours after injection of ACTH. A decrease of less than 50 per cent suggests adrenocortical insufficiency. In adrenal insufficiency secondary to anterior pituitary failure the eosinophils usually also fail to decline because the atrophic adrenal cortex cannot be stimulated sufficiently by a single injection of ACTH; however, in such cases a normal response is elicited by repeated injections of ACTH (4–8 hour test, see below).

False negative tests (i.e., normal response) are obtained at times in subjects with Addison's disease, and false positives (i.e., inadequate response) in hypopituitarism. A decrease of less than 50 per cent is suggestive of Addison's disease, but is not reliable diagnostically.

Determination of the urine uric acid: creatinine ratio after injection of ACTH, which has been proposed as a diagnostic procedure for adrenal insufficiency (i.e., absence of normal 50 per cent increase in ratio), has not proved to be of value clinically.

48-Hour ACTH Test. An initial eosinophil count is obtained as in the four-hour test. Ten milligrams of ACTH are then injected intramuscularly every six hours over a forty-eight-hour period. A second eosinophil count is made 4 hours after the last injection.

Patients with Addison's disease fail to show a significant decrease of eosinophils of 50 per cent or more. The adrenal cortex atrophied due to hypopituitarism will usually be sufficiently stimulated to give a normal response.

If ACTH is given over a period of forty-eight hours, additional information can be obtained by a study of the urinary 17-ketosteroids and/or corticoids. A twenty-four-hour sample is collected prior to the test period, and again on the second day of the test. In Addisonian patients there will be no increase in corticoids or 17-ketosteroids, whereas in hypopituitarism there is usually an appreciable rise from the low excretion levels present before stimulation. An increase of 100 per cent or more may occur in normal subjects.

INTRAVENOUS ACTH TEST. Collect urine for twenty-four hours for estimation of 17-ketosteroids and corticoids (control). Infuse 20–25 units of ACTH in 500 ml. NaCl or glucose solution intravenously continuously over an eight-hour period. Collect urine for the twenty-four hour period following the beginning of the infusion. Eosinophil counts are made at 0 hour, 6 hours, and 10 hours (2 hours after terminating the injection). A normal response includes: (1) a fall in eosinophils of at least 85 per cent at 10 hours; (2) an increase of 10 to 15 mg. in corticoids and 17-ketosteroids.

THYROID HORMONE 1

The chemical nature, biosynthesis, and metabolism of the thyroid hormone are discussed in detail elsewhere (p. 266), as are the diagnostic implications of demonstrable abnormalities in iodine metabolism (p. 270). It will suffice here, therefore, merely to review briefly the metabolic actions of this hormone, and to indicate the various biochemical approaches to the diagnosis of hypo- and hyperthyroidism.

Calorigenic Effect. Thyroid hormone increases the rate of energy exchange and oxygen consumption of all normal tissues except the thyroid gland itself. This is reflected in an increase in the basal metabolic rate (BMR), which is the sum of the effects, of different magnitude, on various tissues.

This calorigenic effect is of smaller magnitude in young than in old subjects and is greater at low (e.g., in hypothyroidism) than at normal or high levels of metabolism. In subjects with myxedema, 1 mg. of thyroxine produces about 2.8 per cent increase in BMR. In the absence of thyroid hormone, the BMR (i.e., resting level of oxygen consumption) decreases by about 30 to 45 per cent (p. 376).

Protein Metabolism. In physiological doses, thyroxine favors protein anabolism, leading to increased retention of nitrogen (positive nitrogen balance). This explains the growth arrest of young thyroidectomized animals and hypothyroid children, and the resumption of growth following administration of thyroid hormone. Large, unphysiological doses stimulate protein catabolism, leading to a negative nitrogen balance.

Thyroidectomy is followed by accumulation of extracellular fluid rich in a hyaluronic acid-containing mucoprotein, apparently similar in composition to fetal mucin. This forms the basis of myxedema in human subjects.

Administration of thyroid hormone is followed by increased urinary excretion of creatine. This may be due in part to increased catabolism of muscle protein and in part to a decreased capacity for synthesizing phosphocreatine by the muscle cell (p. 12).

Carbohydrate Metabolism. Thyroid hormone increases the rate of absorption of monosaccharides by the intestine. It enhances hepatic glycogenolysis, thus increasing the blood sugar concentration. This effect is partially offset by simultaneously increased utilization of glucose in the tissues. There is also evidence that thyroxine enhances gluconeogenesis, but in a manner different from adrenocortical hormone. For example, thyroidectomy, in contrast to adrenalectomy, does not alleviate pancreatic diabetes in the cat or dog, and administration of thyroid hormone, in contrast to adrenocortical hormone, does not increase glycosuria in the Houssay dog (hypophysectomized-depancreatized).

Lipid Metabolism. Fatty acids participate in the increased rate of oxidative processes which follows administration of thyroid hormone. If available stores of carbohydrate are depleted, ketosis develops. However, body fat is not increased in the absence of thyroid hormone.

The state of thyroid function influences the levels of plasma cholesterol and phospholipid, which decrease in hyperthyroidism and increase in hypothyroidism. In the latter state, normal values are restored by administration of thyroid hormone.

Electrolyte and Water Metabolism. Administration of thyroxine to a normal subject is followed by increased urinary excretion of water, potassium, and chloride, indicating mobilization of intracellular fluid. The plasma volume is increased. Hypothyroidism is accompained by an increase in interstitial fluid, and retention of sodium and chloride, the plasma volume being decreased.

In young, growing subjects, small doses of thyroxine enhance calcium retention. This may be secondary to increased formation of bone matrix (osteoid), resulting from stimulation of protein anabolism. Excessive amounts of thyroid hormone lead to increased mobilization of calcium from the skeleton, with increased calcium excretion in the urine and feces. There is no significant alteration in serum calcium.

In the presence of an excess of thyroid hormone, there is an increase in the proportion of "protein-bound" (nondiffusible) magnesium in the blood serum (normal 15–30% of total serum Mg.).

Vitamins. An increase in serum carotene has been observed in the absence of the thyroid. This has been attributed to interference with the synthesis of vitamin A from the provitamin, carotene, presumably in the liver, and may be accompanied by manifestations of vitamin A deficiency. Similar deficiency manifestations may occur in the presence of an excess of thyroid hormone, due presumably to excessively rapid destruction of vitamin A.

Administration of large amounts of thyroid hormone increases the requirement for certain members of the vitamin B complex (thiamine, pyridoxine, pantothenic acid) and for vitamin C. These relationships and the mechanisms involved are obscure.

Hypothyroidism

Basal Metabolism (p. 372ff.). The basal metabolic rate in complete athyreosis is about −40 (−35 to −45). This value is remarkably constant in different individuals. Myxedema occurs when the level of circulating thyroid hormone is low enough to produce a BMR of -30 or less. Hypometabolism of itself is not proof of hypothyroidism. As indicated elsewhere (p. 376), the BMR is low in the majority of cases of Addison's disease except those complicated by active tuberculosis (p. 375); the low BMR in panhypopituitarism is due to absence of thyrotrophin, with consequent secondary hypothyroidism. A low BMR is also found in severe malnutrition. Lowering of the level of oxidation in starvation may be regarded as an economy measure; it is probably mediated by depression of thyrotrophic activity of the anterior pituitary.

The correlation of clinical manifestations with varying degrees of hypometabolism in hypothyroidism can be studied best by suddenly stopping thyroid medication in a patient with complete athyreosis. The BMR decreases and the curve of the readings plotted against the time after cessation of treatment is a parabolic curve which has been called the "decay curve." Over a period of about sixty to eighty days the patient exhibits progressively the symptoms and signs of mild, and later of severe, thyroid deficiency. The strict parallelism between clinical manifestations and metabolic level, although found in the majority of cases, is not always present.

In individuals with a body build which deviates widely from the "norm," it may be impossible to calculate the BMR exactly because of the fact that the conventional standards are invalid. From the clinical standpoint this is particularly important in certain cases of obesity. The amount of inert fat, participating but slightly in tissue oxidative processes, but affecting calculations of body surface area on the basis of body weight, cannot be adequately assessed and, consequently, BMR readings obtained are often "low" when calculated from the usual standard tables.

It would appear that nervous factors can materially depress the BMR. Thus, very low readings (-30 and less) may be encountered in conditions of severe depression. There may be no other clinical or metabolic evidence of hypothyroidism, and the failure to respond to doses of thyroid ordinarily effective is further evidence that the hypometabolism in such cases is not due to depression of thyroid function.

Hypometabolism is also found in perfectly healthy individuals. It would appear that the empirically determined range of "normal" values, indicated by the distribution curve of frequency of BMR readings of -10 to $+10$, may be exceeded considerably in some cases. Values in normal subjects may be as low as -20 to -30. Such observations emphasize the fact that hypometabolism and hypothyroidism are not synonymous. Clinical manifestations and other metabolic changes must be evaluated carefully in order to arrive at a correct diagnosis.

Plasma Cholesterol (p. 117). Plasma cholesterol levels are high in untreated hypothyroidism. Values above 300 mg. per 100 ml. may be considered significant (but not diagnostic). This increase involves both free and ester cholesterol. Phospholipid values are also elevated. However, the serum cholesterol is within the normal range in some untreated hypothyroid or myxedematous subjects. Furthermore, considerable fluctuation of serum cholesterol values has been observed in such patients, greater than in normal individuals. Under adequate treatment the serum cholesterol falls. If thyroid medication is discontinued, an increase is often observed before clinical manifestations of thyroid deficiency are manifest; this rise usually exceeds the pretreatment levels (rebound phenomenon).

Creatine Excretion. Studies of creatine excretion may be of diagnostic value, especially in children. Spontaneous creatinuria (p. 181) and creatinuria following administration of creatine (creatine tolerance test, p. 182) are decreased in hypothyroidism, increasing promptly after thyroid administration. This induced increased creatinuria, which often occurs before other effects of thyroid medication are manifested, has been employed as a diagnostic procedure in children.

Miscellaneous. Serum protein values are frequently elevated; the cerebrospinal fluid protein may be increased.

The fasting blood sugar concentration is within normal limits. Oral administration of sugar (glucose, galactose) results in "flat" types of blood sugar curves (pp. 42, 52), due to a diminished rate of absorption of the sugars from the intestine.

The serum carotene concentration may be elevated, and may contribute to the yellowish color of the skin in myxedema. This may result from inefficient conversion of carotene to vitamin A (p. 456).

The urinary neutral 17-ketosteroids are characteristically low in myxedema (p. 419).

Iodine Metabolism (p. 264). In the majority of cases, the diagnosis of hypothyroidism can be established readily by BMR determination alone, supplemented by the response to thyroid medication. Under circumstances in which basal metabolism cannot be measured readily or satisfactorily, e.g., young children, congestive heart failure, respiratory difficulties, etc., other procedures must be resorted to for the evaluation of the state of thyroid function. The most precise of these involve a study of various phases of iodine metabolism, and have been discussed elsewhere in some detail (p. 270ff.).

The most direct approach is the determination of circulating thyroid hormone as serum "protein-bound" or organic iodine (PBI) (p. 270). This is low in hypothyroidism, i.e., below 4 μg./100 cc. In myxedema it is usually below 2 μg./100 cc. It must be recalled that subnormal values may be obtained in other conditions, e.g., nephrosis, and that spuriously high values may be produced by administration of a variety of iodine-containing compounds.

Where tracer doses of I^{131} are given (p. 272), iodine uptake by the thyroid is subnormal, i.e., at 24 hours less than 10 per cent of the quantity administered. Correspondingly more is excreted in the urine, i.e., over 60 per cent, and usually 90 per cent or more. Moreover, urinary excretion of the I^{131} continues for a longer time than in normal subjects.

The response of the serum PBI and thyroid I^{131} uptake to administration of thyrotrophic hormone (TSH) may at times aid in differentiating between pituitary myxedema and primary myxedema. A response in the direction of normal may be obtained in the former but not in the latter condition.

Hyperthyroidism

Basal Metabolism (p. 374). Characteristically, the BMR is elevated roughly in proportion to the degree of hyperthyroidism. In chronic cases, values between +30 and +60 occur commonly, occasionally as high as +80, rarely higher if sedatives are administered to ensure true basal readings. The occurrence of hypermetabolism in other conditions is discussed elsewhere (p. 372).

The BMR may be within normal limits in hyperthyroidism, particularly in mild cases. It should be remembered that a value of +15 in a subject whose normal BMR is −10 would correspond approximately to a value of +35 in one whose normal BMR is +10.

The main practical limitation of determinations of oxygen consumption in the diagnosis of hyperthyroidism is the fact that a true basal state cannot be attained in the presence of certain common complications, e.g., congestive heart failure (p. 376). The factors of hyperexcitability and apprehension can usually be overcome by the use of sedatives (p. 372).

There has been a tendency to minimize the value of BMR determinations in thyroid functional diagnosis since the introduction of ostensibly more precise methods, such as determinations of serum PBI, thyroid uptake and urinary excretion of I^{131}, etc. However, the fact must not be overlooked that these procedures measure different phenomena than oxygen consumption and that, although they give results that can be interpreted more specifically in terms of thyroid function than can values for oxygen consumption (BMR), they are by no means invariably infallible in this regard. Despite its lack of specificity, its relative insensitivity, the necessity of securing cooperation of the patient, and the several possibilities for errors in technic, the BMR determination remains the most useful single routine laboratory procedure for the evaluation of thyroid function. The use of other, more precise procedures should be restricted to cases in which basal metabolism cannot be measured or in which equivocal or incongruous results are obtained.

Plasma Cholesterol. As stated elsewhere (p. 121), the plasma cholesterol tends to be low in thyrotoxicosis. The diagnostic value of plasma cholesterol levels, however, is diminished by the wide range of normal values. A given value may therefore be well within the normal range and still be low for one individual and high for another; this is true of the great majority of determinations made in cases of thyrotoxicosis. The significantly low values occur in the more severe cases, which usually do not pose diagnostic problems. Whereas a single cholesterol determination is therefore of limited value, serial determinations may be more helpful. If, for example, in a doubtful case the serum cholesterol rises during iodine therapy, this may be regarded as additional evidence of a response typical of thyrotoxicosis.

Carbohydrate Metabolism. Fasting blood sugar values are frequently normal, but in severe thyrotoxicosis they may be moderately elevated, seldom exceeding 150 mg. per 100 cc. Higher values suggest coexisting diabetes (p. 35). Mild intermittent glycosuria is not uncommon.

Oral glucose tolerance tests characteristically reveal a rapid sharp rise in blood sugar (p. 46), the values in the first hour being often 100 per cent or more above the fasting level. This is due in part to an increased rate of absorption from the intestine, and in part to increased hepatic glycogenolysis. The curve, however, in contradistinction to the plateau type of curve seen in diabetes (p. 44), falls rapidly, since peripheral utilization of glucose is increased as a result of increased oxidation. This type of blood sugar curve is seen in severe cases. The practical usefulness of the test is in differentiating glycosuria and mild hyperglycemia due to thyrotoxicosis from coexisting diabetes. However, it is not sufficiently characteristic of thyrotoxicosis to be of diagnostic importance. The same type of glucose tolerance curve may be found in the presence of hepatic disease, among other conditions.

The disturbance of carbohydrate metabolism, and the associated loss of liver glycogen, may be contributed to by a state of B-hypovitaminosis resulting from thyrotoxicosis, unless large amounts of B vitamin, in excess of the normal requirements, are administered. If large amounts of B-vitamins, especially thiamine, are given, carbohydrate metabolism may be improved (p. 459).

Miscellaneous. The protein catabolic action of excess thyroid hormone is reflected in the negative nitrogen balance of thyrotoxic subjects. They may also exhibit spontaneous creatinuria and diminished creatine tolerance (p. 181), but studies in this direction are seldom of diagnostic value.

In severe cases, depletion of carbohydrate reserves and stimulation of fat catabolism may result in ketonemia and ketonuria. This may pose a diagnostic problem if accompanied by glycosuria. However, they are usually of mild degree, and, in contradistinction to diabetic ketosis, do not lead to severe acidosis.

Skeletal demineralization in severe thyrotoxicosis is associated with negative Ca balance, i.e., excessive loss of Ca in the feces and urine. The serum Ca remains within normal limits.

Iodine Metabolism. Characteristically, in hyperthyroidism, abnormalities in thyroid function are reflected in certain aspects of iodine metabolism, including the following: (*a*) increase in serum organic iodine (PBI), i.e., above 8 μg./100 cc. (p. 270); (*b*) increased uptake of I^{131} by the thyroid, i.e., more than 40 per cent of the tracer dose present after 24 hours (p. 272); (*c*) decreased urinary excretion of a tracer dose of I^{131}, i.e., less than 40 per cent in 24 hours (p. 272); (*d*) increased "conversion ratio" of I^{131}, an index of the rate of release of thyroid hormone into the circulation (p. 273).

These are considered in detail elsewhere and require no elaboration here. The fact should be emphasized that these methods are by no means invariably infallible in thyroid functional diagnosis. They are most useful clinically in the presence of difficult diagnostic situations in which the BMR is difficult or impossible to obtain or in which the values obtained are equivocal or cannot be reconciled with the clinical findings.

PARATHYROID HORMONE

The clinically pertinent aspects of parathyroid physiology and of the metabolic actions of the parathyroid hormone are discussed in detail in the section on calcium and phosphorus metabolism. There are no satisfactory procedures for direct assay of parathyroid hormone in body fluids that are applicable clinically. Evaluation of the state of parathyroid function must depend, therefore, on studies of aspects of Ca and PO_4 metabolism that are known to be influenced by this factor.

Accurate diagnosis of hypo- and hyperparathyroidism is usually accomplished quite readily by studies of Ca and PO_4 concentrations and alkaline phosphatase activity in the blood, and Ca and PO_4 excretion in the urine. These are described in detail elsewhere (pp. 233, 236) and need merely be summarized briefly here.

The characteristic, indeed almost pathognomonic metabolic manifestations of uncomplicated hyperparathyroidism are: (*a*) hypercalcemia, (*b*) hypophosphatemia, (*c*) negative Ca and PO_4 balances (increased urinary excretion, and (*d*) increased serum alkaline phosphatase activity. These findings may be altered in the presence of complicating renal disease or urinary obstruction (p. 233).

The characteristic metabolic manifestations of hypoparathyroidism are: (*a*) hypocalcemia, (*b*) normal or elevated serum PO_4 concentration, (*c*) positive Ca and PO_4 balances, (*d*) normal alkaline phosphatase activity, and (*e*) normal acid-base balance.

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CHAPTER XVIII

Vitamins

Although procedures are available for quantitative determination of most of the known vitamins, especially in foods, clinical evaluation of nutritional status with respect to vitamins is often difficult and the available methods unsatisfactory. This is particularly true during the usually long latent period of progressing deficiency which precedes the appearance of frank symptoms and signs. Various types of diagnostic procedures may be employed for this purpose:

1. The concentration of the vitamin or one of its metabolites may be determined in the blood or urine.
2. The curve of concentration in the blood or excretion in the urine may be measured after administration of a standard test dose of the vitamin. This is the so-called "saturation test" procedure, based on the assumption that subsaturation of the tissues with the vitamin will result in a subnormal rise in the blood and subnormal excretion in the urine under conditions of the test.
3. Quantitative determinations may be made of the vitamin content of tissues obtained at biopsy (e.g., liver, muscle).
4. Evidence of certain types of deficiency may be obtained by microscopic studies, e.g., of mucosal scrapings in vitamin A deficiency.
5. Certain consequences of specific deficiencies may be demonstrated by biophysical methods, e.g., impaired dark adaptation in vitamin A deficiency and increased capillary fragility in ascorbic acid deficiency.
6. In the case of certain vitamins, deficiency may manifest itself in some characteristic derangement of metabolism which can be measured quantitatively, e.g., elevation of blood pyruvate in thiamine deficiency, and increase in serum alkaline phosphatase, hypophosphatemia, and hypocalcemia in vitamin D deficiency.
7. Of great diagnostic value is the prompt relief of clinical manifestations of suspected deficiency upon administration of adequate amounts of the vitamin in question.

We are concerned here chiefly with quantitative estimations of the vitamins or their metabolic products in the body fluids. Such methods are available at present for only a few of the known vitamins. Moreover, they have distinct limitations in their applicability to the appraisal of nutritional status. Inasmuch as the blood is a labile transport medium, its vitamin content in chronic deficiency states may be increased after a comparatively brief period of high vitamin intake without comparable improvement in the morphologic abnormality in the tissues. In the evolution and recession of a state of avitaminosis, changes in concentration of the vitamin in the blood and urine do not occur synchronously with alteration in the tissue state. The same may be said of the so-called "saturation tests," although results obtained by these procedures are perhaps not subject to as rapid fluctuation as the blood values. The extent of the tissue changes, indicating the severity of the deficiency state, can be best determined at times by biomicroscopy. In certain instances, however, rather prolonged deficiency is required for the production of morphologic abnormalities, and blood or urine studies or saturation tests may yield earlier abnormal results. In established chronic deficiency states, which are the type encountered most frequently clinically, blood and urine studies may be misleading, particularly during periods of specific therapy.

There is considerable evidence that the immediately antecedent dietary intake of a vitamin is an important factor in determining the level at which it will be excreted in the urine. It is therefore possible that a high excretion level may result from a recent but transient high intake and a low excretion level from a recent but transient low intake. Such excretion studies might lead to erroneous interpretations of the nutritional state with regard to the factor under investigation. This has been demonstrated to be true in the case of niacin, riboflavin, pyridoxine, pantothenic acid, folic acid, biotin and choline. A lack of correlation was found between the biochemical data and the results of tests of physical (bicycle ergometer) and psychomotor (pursuit meter) performance tests. On the basis of these observations, the conclusion seems justified that biochemical data of this type, although important in nutritional appraisal, are most valuable when used in conjunction with other types of data, e.g., clinical, dietary, physical and psychomotor. Any single type of study, employed alone, may be misleading.

Only those vitamins are considered here which at the present time lend themselves to reasonably accurate quantitative determination in biological fluids as an aid in the detection of nutritional deficiency states, or which influence metabolic processes in such a manner that diagnostically significant biochemical abnormalities are produced as a result of deficiency.

VITAMINS A

There is little substantial evidence that vitamin A deficiency is accompanied by any significant disturbance of protein, fat or carbohydrate metabolism. There have been isolated reports of marked increase in the esterase content of the blood serum in vitamin A deficient rats and of an increase in serum cholesterol following administration of excessive amounts of vitamin A. In the growing dog with vitamin A deficiency there is increased osteoblastic and osteoclastic activity, with proliferation of cancellous bone at the expense of compact bone, the overgrowth of bone causing compression of adjacent nerve fibers and cells, with consequent changes in the central nervous system. This factor may also be necessary for normal development of the teeth. Visual acuity in dim light is dependent upon the presence in the rod cells of the retina of an adequate amount of a photosensitive pigment, rhodopsin or visual purple, a dissociable combination of a protein, "opsin," and vitamin A aldehyde (retinene).

Vitamin A and provitamin A, carotene, are absorbed from the intestine, bile salts being necessary for absorption of the latter. Carotene is apparently absorbed largely into the portal circulation and vitamin A chiefly by the lymphatics into the thoracic duct. Absorption of vitamin A is impaired in the absence of pancreatic enzymes (impaired hydrolysis of vitamin A esters) and in celiac disease or other conditions in which there is impaired absorption of dietary fat (e.g., obstructive jaundice, chronic pancreatitis). The liver is apparently the chief site of conversion of carotene to vitamin A as well as the chief site of storage of the latter, which is mobilized in response to physiologic demands. There is evidence that the thyroid gland and also the intestinal wall (in certain species) may be of major importance in the conversion of carotene to vitamin A. Ingestion of alcohol, sympathicoadrenal stimulation or administration of certain carcinogenic polycyclic hydrocarbons (e.g., benzanthracene) result in active mobilization of vitamin A from the liver, with a rise in its concentration in the blood.

About 95 per cent of the vitamin A reserves of the body is held in the liver, chiefly in ester form (combined with fatty acids), a small amount being present in other tissues, e.g., lactating breast, adrenals, lung, intestine. Under normal conditions, vitamin A is stored chiefly in the liver cells; after ingestion of large amounts it enters the Kupffer cells also. In subjects with liver damage, the capacity for storage and formation of vitamin A from carotene is impaired and concentration of this vitamin in the blood is decreased. The hepatic storage capacity is comparatively low in young infants, increasing with age. The quantity stored in the liver varies in different species, but is largely dependent upon the antecedent diet. About 70 per cent of a single large dose of vitamin A may be recovered from the liver of the rat, which can store enough in a few days of adequate intake to satisfy its requirement for months. On the other hand, less than 10 per cent of a similar dose can be recovered from the liver of the guinea pig, which is very sensitive to deficiency in vitamin A. The storage capacity in man is apparently relatively large.

The blood plasma contains both vitamin A and carotenoid pigments. The former is present chiefly in the free state except after ingestion of large amounts of vitamin A or its carotene precursors, when the ester form appears in the blood. Under normal conditions, the levels of both substances in the blood are maintained by transformation of carotene to vitamin A in the liver and mobilization of the latter from the hepatic stores. The mechanism which regulates this mobilization is not clear, but certain factors are known to influence it.

Under conditions of decreased intake the plasma vitamin A concentration is maintained at the expense of the hepatic reserves. Hyperthermia causes depletion of liver vitamin A with a simultaneous decrease in its concentration in the blood. Administration of thyroxine or testosterone accelerates mobilization from the liver, whereas 17-α-hydroxyallopregnane-20-one, an adrenal steroid, produces a decrease in the plasma and a simultaneous increase in the liver. These observations suggest that certain steroid hormones may regulate the equilibria between liver and plasma vitamin A or between the esterified and unesterified forms in the liver.

Reported values for vitamin A and carotenoids in the blood of normal subjects vary widely, owing in part to differences in methods employed, several of which have proved unsatisfactory. The following figures appear to be acceptable:

vitamin A, 18 to 60 μg. (60–200 I.U.) per 100 cc. serum; carotenoids, 100 to 300 μg. per 100 cc. serum.

The plasma vitamin A concentration is usually but not invariably subnormal in subjects maintained on a diet deficient in vitamin A, and, although there may be a general parallelism between the intake and the concentration in the blood, the value of blood vitamin A levels in assessing nutritional status in this connection is uncertain. Subnormal plasma values have been obtained also in conditions mentioned above in which absorption from the intestine is impaired, in gastro-intestinal malignancy and in a number of types of hepatic disease. No definite correlation has been established between the results of biophotometric studies and the vitamin A content of the blood, which apparently bears no constant relation to the adequacy of the vitamin stores in the body. However, the statement has been made, on the basis of an extensive study of this problem, that a high value for vitamin A in the blood is inconsistent with deficiency of this vitamin. Low values for vitamin A and carotene have been obtained in hepatitis and cirrhosis of the liver.

The urine contains no vitamin A or carotene except after administration of excessive amounts. Under normal conditions only very small quantities are excreted in the feces. Administration of mineral oil, especially in young children, may cause excessive loss of carotene in the feces. Considerable amounts of vitami A and carotene are present in milk, the concentration being greatest in colostrum and decreasing gradually over the period of lactation. Human colostrum possesse about twice as much vitamin A activity as early milk, the latter, providing 240 to 4000 I.U. daily, being considerably richer in this factor than cow's milk. Th vitamin A content of milk is increased by ingestion of added amounts of vitamin during pregnancy, but not by similar doses of carotene.

Deficiency in Man. Present understanding of the role of vitamin A i human nutrition is reflected in the following statements (A.M.A. Council o Pharmacy):

1. Vitamin A is specific for the cure and prevention of xerophthalmia nyctalopia (night blindness), and hemeralopia (day blindness).
2. Vitamin A is essential to the normal structure and behavior of epitheli tissue, e.g., the epithelium covering the skin, and forming the lining of the nas sinuses and respiratory tract, mouth, pharynx, entire digestive tract, and th genitourinary tract. It prevents follicular hyperkeratosis of the skin.
3. Vitamin A is a growth factor.

Detection of Deficiency in Man. The most important clinical manifest tions of vitamin A deficiency in man are: (1) xerophthalmia, i.e., thickening an loss of transparency of the bulbar conjunctiva, with yellowish pigmentation an occasionally Bitot's spots; (2) follicular conjunctivitis; (3) keratomalacia, i.e softening of the cornea with, in advanced cases, ulceration and necrosis; (4) im pairment of dark adaptation, progressing to night blindness (nyctalopia (5) follicular hyperkeratosis of the skin.

The clinical manifestations and their prompt response to administration adequate amounts of vitamin A constitute the best available means of diagnos

No reliable procedures are available for detecting subclinical deficiency states. The following objective methods have been investigated: (1) determination of the concentration of vitamin A and carotene in the blood; (2) the dark adaptation test; (3) examination of scrapings of the bulbar conjunctiva and vagina (and other mucous membranes).

Vitamin A Content of Blood. The concentration of vitamin A in the blood (plasma or serum) is not a reliable index of the status of vitamin A nutrition, although extremely low values (below 40 I.U./100 cc.) may be regarded as indicative of deficiency. One source of difficulty is lack of agreement as to the limits of normal concentration. Of greater importance, however, is the fact that a normal plasma vitamin A level may be maintained for many months in previously normal subjects receiving diets containing virtually no carotene or vitamin A. There is considerable individual variation in this connection, due undoubtedly to quantitative differences in the hepatic stores of the vitamin which, in well-nourished, normal subjects may be adequate to meet the body requirements for several months. Fever may be accompanied by a sudden drop in plasma (and liver) vitamin A, and a rise follows administration of large doses of the vitamin. There is no satisfactory consistent correlation between the blood vitamin values and the presence or severity of the various clinical deficiency manifestations. The plasma carotene level is of still less diagnostic value. It reflects the immediate past intake of carotenoids, falling promptly after their exclusion from the diet and rising promptly following their administration. A zero value is compatible with normal nutrition, inasmuch as there is no consistent relationship between the level of plasma carotene and the quantity of vitamin A in the organism.

Dark Adaptation Test. The biophotometer has been employed to test the speed of visual adaptation to dim light after a period of exposure to bright light. Although night blindness is an important manifestation of vitamin A deficiency, the general experience has been that the dark adaptation test does not satisfactorily reflect subclinical levels of vitamin A deficiency and that biophotometer readings do not parallel the concentration of the vitamin in the blood. The time of development of significant impairment of dark adaptation in subjects on a diet low in vitamin A and carotenoids varies widely, from several days to many months. Moreover, conditions other than vitamin A deficiency may cause this phenomenon.

Examination of Scrapings. In established A-deficiency, keratinized epithelial cells may be demonstrated in scrapings from the bulbar conjunctiva and the vagina. These changes generally occur rather late, being preceded usually by subjective evidence of impaired dark adaptation. This procedure is therefore of little clinical value.

Effects of Excess of Vitamin A. Acute symptoms may follow ingestion of very large amounts of vitamin A. These include drowsiness, sluggishness, severe headache, vomiting, and peeling of the skin about the mouth and elsewhere. This syndrome has been recognized by Eskimos as occurring after eating the livers of polar bears and arctic foxes, which are extremely rich in vitamin A.

Continued intake of excessive amounts, especially in children, produces roughening of the skin, irritability, coarsening and falling of the hair, anorexia, loss of weight, headache, vertigo, hyperesthesia, occasionally hepatomegaly, splenomegaly, hyperlipemia and hemorrhages (in some species), and certain rather characteristic skeletal changes. The hemorrhagic manifestations are associated with hypoprothrombinemia, and may be prevented by simultaneous administration of vitamin K. This effect may be due to interference with bacterial synthesis of vitamin K in the intestine.

The skeletal changes are the result chiefly of acceleration of the normal bone growth sequences, with simultaneous increase in the processes of resorption (osteoclasis) and cortical bone deposition (hyperostosis). Young rats and guinea pigs fed the large amounts of vitamin A may show an equivalent of a year's growth in a few weeks, the new bone being inadequately mineralized and fracturing easily. Weanling rats fail to grow, have difficulty in walking and pain in the extremities, with fractures and associated hemorrhages.

Infants may tolerate daily doses of about 200,000 I.U. of vitamin A for a year, but develop symptoms with 500,000 I.U. daily for even brief periods. On this basis the toxic daily dose for adults would be between 1,000,000 and 3,000,000 I.U.

Carotenemia. This is a condition of yellow pigmentation of the blood plasma and selected areas of the skin, due to an increase in carotenoid pigments (carotene). The occurrence of this condition in diabetes (xanthosis diabetica) and in malnourished children was attributed by many to ingestion of an excessive amount of lipochrome-containing vegetables. That this is not the fundamental cause is shown by the difficulty of production of the condition in healthy subjects by this means.

Carotenemia may occur in diabetes mellitus, advanced chronic glomerulonephritis, myxedema, acute hepatic disease, and malnutrition with ingestion of unusual quantities of vegetables rich in carotenoid pigments. It may be responsible in part for the yellowish or sallow discoloration of the skin in these conditions. It is significant, perhaps, that the clinical disorders in which this condition is prone to develop are also commonly accompanied by lipemia.

It has been suggested that carotenemia (fasting plasma carotene more than 400 micrograms per 100 cc.) occurs when the quantity of carotene reaching the liver exceeds its capacity for storage or conversion of this substance to vitamin A. Some observers have found that the blood carotene curve after ingestion of carotene in oil is higher and the rise more prolonged in diabetics than in normal subjects. It was suggested that this may be due to impaired formation of vitamin A from carotene (hepatic dysfunction). These findings have been contradicted by other workers.

Carotenemia may be confused with hyperbilirubinemia if reliance is placed on the icterus index determination alone (p. 521), which is elevated in this condition. The serum bilirubin concentration is normal in uncomplicated carotenemia and the diagnosis is established by determination of the serum carotenoid (carotene) concentration.

THIAMINE

Thiamine, in the form of the pyrophosphate, the active form of this vitamin, is the cofactor (coenzyme) in reactions involving oxidative decarboxylation of certain important intermediates in carbohydrate metabolism, e.g., pyruvic and α-ketoglutaric acids (p. 8). It is therefore referred to as "cocarboxylase." The biochemical and probably also the clinical manifestations of thiamine deficiency are related to the consequent defect in this vital phase of intermediary metabolism.

Metabolism of Thiamine. Free thiamine is absorbed readily from the intestine, but the pyrophosphate (cocarboxylase) is not. The bulk of the dietary vegetable thiamine is in the free state. It is very actively phosphorylated to cocarboxylase in the liver and, to a lesser extent, in other tissues (muscle, brain), including nucleated red blood cells.

It is present in the blood plasma and cerebrospinal fluid in the free state (about 1 µg./100 cc.). The largest portion of the blood thiamine, which ranges from 6 to 12 µg./100 cc., is in the blood cells as the pyrophosphate, in protein combination. Since free thiamine (base) is readily diffusible and the pyrophosphate is not, the plasma thiamine probably represents the transport form (inactive) of the vitamin, which undergoes phosphorylation (activation) upon entrance into tissue cells, including nucleated red blood cells in the bone marrow.

The capacity of the organism for storing thiamine in any form is apparently limited. It is present in both free and combined forms, mainly the latter, in the heart, liver, and kidneys, and, in lower concentration, in skeletal muscle and brain. Administration of thiamine may result in an increase in the tissues, within certain limits. However, on a thiamine-free diet, the tissue content is depleted within a short time, emphasizing the desirability of providing an adequate daily supply.

If normal amounts of thiamine are ingested (1–2 mg. daily), about 10 per cent is excreted in the urine. The remainder is apparently partly phosphorylated and utilized for carboxylase action and partly degraded to neutral sulfur compounds and inorganic sulfate. If large amounts are given, the excess is largely excreted in the urine. All of the urinary thiamine is in the free form. Normal subjects on an adequate intake excrete at least 50 µg. daily in the urine. That present in the feces is probably largely of bacterial origin (large intestine).

Demonstration of Deficiency. The important biochemical features of human thiamine deficiency include: (*a*) decreased levels of thiamine and thiamine pyrophosphate in the blood and urine; (*b*) increased concentrations of pyruvic and lactic acids in the blood; (*c*) decreased amounts of thiamine in the tissues. Several procedures have been proposed for the laboratory diagnosis of deficiency states, none of which is entirely satisfactory.

Thiamine in Blood. Some believe that values below 3 micrograms of thiamine per 100 cc. of whole blood are indicative of deficiency in this factor, subnormal levels having been associated with the development of peripheral neuropathy in alcohol addicts. It has been found that the thiamine content of the leukocytes reflects states of thiamine deficiency and saturation more satisfactorily than does the thiamine content of whole blood. High thiamine values (three times normal in leukocytes, two times normal in erythrocytes) have been obtained in patients with leukemia, Hodgkin's disease and carcinoma of the gastrointestinal tract. This has been attributed to impaired utilization of thiamine.

After intravenous injection of 50 mg. of thiamine, the level in the serum rises from a control concentration of 0 to 2 micrograms per 100 cc. to a peak of 130 to 200 micrograms at five minutes, falling sharply subsequently to 5 to 15 micrograms at one hour. The rise is greater and more sustained in subjects receiving thiamine, due perhaps to repletion of the body stores of the vitamin.

Thiamine Excretion. Normal limits of excretion of thiamine are difficult to establish because alterations in intake are reflected promptly in its excretion in the urine. Reported values vary also with the method employed, fermentation methods generally yielding higher values than colorimetric procedures and the latter higher results than the rat bradycardia method. The daily urinary excretion by normal subjects receiving adequate amounts of thiamine ranges usually from 60 to 500 micrograms; however, values as low as 25 micrograms have been reported, while some believe that the excretion of less than 90 micrograms is evidence of inadequate intake.

More satisfactory information regarding the state of thiamine nutrition may be obtained by determining the quantity excreted in the urine after administration of a standard test dose. Several procedures have been suggested. Intravenous injection of 1 mg. of thiamine is followed, in normal adults, by an increment of not less than about 110 micrograms in the urine in the next twenty-four hours, almost all of which appears in the first half-hour. In children under two years more than 250, and from two to five years more than 150 micrograms of the injected thiamine are excreted in twenty-four hours. Very low pre-injection values do not necessarily indicate disease, but may be caused by temporary reduction in thiamine intake. At least 6 per cent of the test dose should be excreted in the urine within four hours after injection of 350 micrograms of thiamine per square meter of body surface. It has been found that after intravenous injection of 1 mg. in normal subjects, free thiamine constitutes more than 60 per cent of the yeast-fermenting substances excreted in the urine during the next twenty-four hours. In a study of unselected hospital patients, 25 per cent excreted less thiamine than other pyrimidines that accelerate yeast fermentation (*PAYF*). It is suggested that low thiamine and normal *PAYF* excretion under these conditions indicates recent thiamine deprivation, while subnormal values for both factors indicate protracted deficiency.

On an intake of 0.4 mg. thiamine per 1000 calories, normal subjects excrete at least 18 per cent of the test dose of 1 mg. (intramuscular injection) in the urine in twenty-four hours. Decreased excretion with this procedure was found to be the earliest indication of thiamine deficiency in two otherwise normal subjects in whom deficiency was induced by restriction of diet.

When 0.1 mg. of thiamine per kilogram of body weight is taken by mouth, 8 to 10 per cent of the test dose is excreted normally in the urine in twenty-four hours, most of it in the first five hours.

These "saturation" tests have yielded subnormal results in a variety of clinical conditions other than frank thiamine deficiency, including thyrotoxicosis, various neuropathies, diseases of the central nervous system, diarrhea and other gastrointestinal disorders, congestive heart failure, deficiency in vitamins other than thiamine, and chronic diseases of various types. Variable findings have been reported in diabetes. Impaired renal function may result in subnormal excretion in the urine in the absence of thiamine deficiency.

Blood Pyruvic and Lactic Acids. In the fasting state, normal blood plasma contains 0.4 to 2 mg. of pyruvic acid and 5 to 20 mg. of lactic acid per 100 cc. These are increased in thiamine deficiency, but also in other conditions, e.g., congestive heart failure. It has been shown that in normal subjects the ingestion of glucose is followed by a short, steep elevation in blood pyruvic and lactic acids, which reaches a maximum in one hour and returns to the resting level in three hours. The curve is abnormally high and prolonged in subjects with thiamine deficiency. Before a significant increase occurs in the basal pyruvic acid concentration in deficiency states, intravenous injection of 0.4 gm. of glucose per kilogram of body weight in 50 per cent solution is followed by an abnormally high rise in blood pyruvic acid and lactic acid. On the basis of this abnormal response to carbohydrate in thiamine deficiency, a "carbohydrate metabolic index" has been evolved, which has a value of 5 to 10 for normal subjects and is greater than 10 in those with thiamine deficiency. This phenomenon usually develops some time after "saturation" studies yield abnormal results.

RIBOFLAVIN

Riboflavin, in the form of mono- and dinucleotides, is a coenzyme in oxidation-reduction reactions, which are intimately concerned with a number of vital metabolic processes. These enzymes serve as bridges over which hydrogen atoms pass from one molecule to another.

Human blood plasma contains 2.5 to 4.0 μg. of riboflavin per 100 cc., about two-thirds as the dinucleotide, the bulk of the remainder as the mononucleotide. The concentration in erythrocytes has been reported as 15 to 30 μg./100 grams, and in leukocytes (plus platelets) as about 250 μg./100 grams. These values remain quite constant even in severe riboflavin deficiency. Determination of riboflavin in the blood is not useful in the clinical evaluation of the state of riboflavin nutrition.

Riboflavin is present in all tissue cells, principally as the nucleotides (coenzymes), a variable proportion of which is bound as flavoprotein. The retina apparently contains free riboflavin. The animal organism does not appear to have a specialized mechanism for storage of riboflavin. The highest concentrations occur in the liver and kidneys, but the tissue content is not increased significantly by administration of large amounts. Certain tissues (e.g., muscle) may retain considerable quantities in the presence of manifestations of riboflavin deficiency.

Riboflavin is secreted in the milk, 40 to 80 per cent being in the free state, increasing with increased intake. It is also present in perspiration (10 μg./hour). The riboflavin content of the feces (free and nucleotides) tends to remain quite constant (500-750 μg. daily), and is presumably largely of bacterial origin. The urinary excretion (mainly free, but up to 50 per cent nucleotide) varies with the intake. Under ordinary dietary conditions (1–2 mg. riboflavin), the daily urinary excretion is about 0.1 to 0.4 mg. (10–20% of intake). When larger amounts are administered, as much as 50 per cent may be eliminated in the urine. The bulk of the dietary riboflavin is metabolized in the body, largely to unknown compounds; a substance of undetermined constitution, uroflavin (aquoflavin), present in the urine, is believed to be a degradation product of riboflavin.

Demonstration of Deficiency. As is true of other B vitamins, demonstration of riboflavin deficiency in man rests mainly on the prompt clinical improvement that follows administration of adequate amounts of the vitamin.

Determination of the blood level or daily urinary excretion of riboflavin has not proved to be of diagnostic value, as indicated above. Saturation tests may be useful if adequately standardized. Normal subjects excrete at least 20 per cent of a test dose of 3 mg. during the subsequent twenty-four hours. Less is excreted in riboflavin deficiency.

NIACIN (NICOTINIC ACID)

Nicotinic acid occurs principally as the amide (nicotinamide; niacinamide), in which form it enters into the formation of the physiologically active di- and trinucleotides. These, like the riboflavin nucleotides, serve as coenzymes in fundamental oxidation-reduction reactions, i.e., as a medium for the transfer of hydrogen from one molecule to another.

Certain tissues and bacteria can synthesize niacin from the amino acid, tryptophan. Consequently, the dietary supply of this vitamin is supplemented by its tissue and intestinal bacterial synthesis in the presence of adequate provision of proteins rich in tryptophan.

Nicotinic acid and its amide are absorbed from the intestine, the concentration in the blood plasma rising promptly after oral administration of large doses (20 mg.). Stated values for human blood are as follows: (1) whole blood, total nicotinic acid activity, 0.2 to 0.9 (av. 0.6) mg./100 cc. (2–3.5 [av. 3.0] mg. as coenzyme); (2) erythrocytes, total nicotinic acid activity, 1.3 mg./100 cc. (6.5–9.0 [av. 7.0] mg. as coenzyme); free nicotinic acid, 0.135 mg./100 cc.; (3) plasma, total nicotinic acid activity, 0.025 to 0.15 (av. 0.075) mg./100 cc. Most of the nicotinic acid (or amide) in the blood is in the erythrocytes, mainly in combination, presumably as coenzyme. That in the plasma is apparently largely in the free state. The values in the blood are not altered significantly in severe niacin deficiency (i.e., in pellagra), and their determination is therefore of no value in the detection of clinical deficiency states.

Normal adults, on a normal diet, excrete both nicotinic acid and nicotinamide in the urine (0.25–1.25 mg., and 0.5–4 mg. daily, respectively). However, the major urinary metabolite is a methylated derivative, N1-methylnicotinamide, also referred to as F_2 (3.0–12.5 mg. daily), which exhibits a bluish-white fluorescence in alkaline butanol in the ultraviolet. There also may be variable amounts of an oxidation product of the latter. Urinary excretion of the latter substance accounts for 40 to 50 per cent and of F_2 for about 10 to 20 per cent of administered nicotinamide. These processes of methylation and oxidation occur in the liver. In rats, administration of large amounts of nicotinic acid or nicotinamide may produce fatty liver, which is prevented by simultaneous administration of methionine, choline, or betaine (p. 109). This phenomenon is apparently due to diversion of methyl groups for the formation of N^1-methylnicotinamide.

Traces of nicotinamide are present in the sweat. Small amounts are secreted in human milk, increasing from less than 0.05 mg. on the first day postpartum to about 3.0 mg. on the tenth day (intake 16.5 mg. daily). Somewhat larger amounts are present in cow's milk.

Demonstration of Deficiency. In its fully developed form the clinical picture is rather characteristic and response to administration of adequate doses of nicotinic acid or nicotinamide is prompt and dramatic. The diagnosis is usually made easily on the basis of clinical features and response to specific therapy.

Available diagnostic laboratory tests, although useful, are not entirely reliable. The most promising are based on the "saturation" principle, in which the urinary excretion of N^1-methylnicotinamide is measured following administration of a standard test dose of nicotinic acid or nicotinamide. The amount excreted is subnormal in niacin deficiency. As is true of most "saturation tests," difficulty is encountered chiefly in the establishment of normal standards.

ASCORBIC ACID

The fundamental role played by ascorbic acid in metabolic processes is not known. The fact that it is very sensitive to reversible oxidation (ascorbic acid $\rightleftharpoons$ dehydroascorbic-acid) suggests that it may be involved in cellular oxidation-reduction reactions. However, there is no direct evidence that this is the case. There is evidence that ascorbic acid may be involved in the intermediary metabolism of tyrosine and in the conversion of folic acid to the citrovorum factor (folinic acid).

Ascorbic acid is essential for the normal regulation of the colloidal condition of intercellular substances, including the fibrils and collagen of connective tissue, osteoid tissue, dentin, and perhaps the intercellular "cement substance" of the capillaries. Many of the important clinical manifestations of vitamin C deficiency are directly dependent upon abnormal development and maintenance of these structures. The capillary defect in scurvy may be related to an inhibitory effect of ascorbic acid on the hyaluronidase-hyaluronic acid system.

Only man, other primates and guinea pigs, of the many species investigated, are unable to synthesize ascorbic acid, the entire human requirement for which must consequently be supplied by the diet. Ascorbic acid is absorbed readily from the small intestine, peritoneum, and subcutaneous tissues. It is widely distributed throughout the body, in local concentrations roughly paralleling the metabolic activity of the tissue. Ascorbic acid is supplied to the fetus from the maternal circulation, passing the placental barrier readily. The concentration in umbilical cord blood is higher than in the maternal blood, suggesting that the placenta may be able to concentrate the vitamin.

There is no evidence that any particular organ or tissue serves as a storage reservoir. That the body does contain mobilizable reserve stores of ascorbic acid is indicated, however, by the fact that clinical manifestations of scurvy develop in man only after several months on an ascorbic acid-free diet.

Normal human blood plasma contains about 0.6 to 1.5 mg. ascorbic acid per 100 cc. Under adequate dietary conditions the concentration in the erythrocytes is one to two and one-half times, and in the white blood cells and platelets ("white layer") twenty to forty times that in the plasma.

The vitamin exists in the body largely in the reduced form (ascorbic acid), in reversible equilibrium with a relatively small amount of dehydroascorbic acid (oxidized form). Under conditions of normal dietary intake (75–100 mg.), about 50 to 75 per cent of ingested ascorbic acid undergoes metabolic conversion to inactive compounds. The remainder is excreted, as such, in the urine. It is also present in milk in active form. The main catabolic pathway is degradation to CO_2. The other demonstrated important metabolic end-product is oxalic acid, which is eliminated in the urine.

Detection of Deficiency in Man. Advanced scurvy is readily diagnosed, but is rarely seen. Detection of subclinical deficiency is difficult, as is demonstration of ascorbic acid deficiency as a cause of various clinical conditions. As in the case of vitamin deficiencies generally, prompt improvement following administration of ascorbic acid constitutes the most reliable evidence that deficiency in this substance had contributed to the development of the condition in question. Certain objective methods have been proposed for the demonstration of subclinical deficiency states. They include: (1) the concentration of ascorbic acid in the blood; (2) urinary excretion of ascorbic acid; (3) urinary excretion following administration of the vitamin (saturation test); (4) intradermal test for ascorbic acid; (5) capillary fragility test.

BLOOD ASCORBIC ACID. The plasma ascorbic acid concentration falls relatively promptly after removal of the vitamin from the diet, approaching zero in forty to eighty days. Usually, when the plasma values fall below 0.3 mg./100 cc. the concentration in the leukocytes and platelets begins to decrease, and when the former reaches 0.1 mg./100 cc. the latter may be assumed to be seriously reduced. As a rule, recognizable clinical manifestations of scurvy appear only after this degree of depletion has been reached. Consequently, decrease in ascorbic acid of the "white blood cell-platelet layer" or, less significantly, of whole blood, is a more reliable index of the scorbutic nature of clinical signs than is a fall in the plasma ascorbic acid concentration.

According to some observers, plasma values above 1 mg. per 100 cc. may be regarded as indicating normal vitamin C saturation, and values below 0.6 mg. undersaturation. However, values below 0.4 mg. are obtained so frequently in apparently healthy subjects that a diagnosis of subclinical vitamin C deficiency based upon this finding alone is questionable. The plasma ascorbic acid concentration reacts almost immediately to variations in intake and, therefore, much more reliance is to be placed upon the concentration in whole blood or the white cell-platelet layer. In a normal subject given a diet deficient in vitamin C, ascorbic acid fell to zero in the plasma after 41 days, and in the whole blood and "white layer" after 124 days, the first clinical signs of scurvy appearing on the 134th day. Thus, the plasma is the first and the "white layer" the last to be depleted, the latter being apparently the most sensitive index of the state of vitamin C nutrition. It has been suggested that a diagnosis of vitamin C deficiency is warranted when the plasma ascorbic acid concentration is zero, and a diagnosis of a prescorbutic state when the whole blood ascorbic acid is zero.

URINE ASCORBIC ACID. Urinary excretion of ascorbic acid decreases rather promptly following lowering of the intake and does not therefore reflect the nutritional status of the organism in this regard. Test procedures which embody the "tissue saturation" principle are perhaps more useful clinically. These saturation tests are based upon the principle that if the tissues contain an adequate amount of ascorbic acid, i.e., are "saturated" with the vitamin, a certain proportion of an administered test dose will be excreted in the urine. If the tissues are "undersaturated" they will retain an abnormally large proportion of the test dose, and a subnormal amount will be excreted in the urine. A number of different procedures and dosages have been employed, with rather equivocal results. The chief difficulty has been in the establishment of normal values because of the wide range of individual variation. Such saturation tests may be valuable under carefully controlled conditions.

Normal adults receiving an adequate amount of vitamin C usually excrete 20 mg. or more daily in the urine. In clinical scurvy there is no ascorbic acid in the urine, the traces reported occasionally probably consisting of other reducing substances. The twenty-four-hour excretion of ascorbic acid is a poor index of vitamin C deficiency, being dependent upon the degree of saturation of the tissues with regard to this factor. This is due to the fact that in the "unsaturated" subject about 99 per cent of the ascorbic acid presented to the kidneys is reabsorbed in the tubules, whereas in the "saturated" subject all the excess of the vitamin ingested above that which can be utilized is excreted.

A more satisfactory index of the state of vitamin C nutrition is afforded by the use of the saturation test, involving measurement of the urinary excretion of ascorbic acid following administration of a test dose. The interpretation of this test is based upon the hypothesis that if the vitamin C content of the tissues is subnormal an abnormally large proportion of the administered dose will be retained in the body and, consequently, a smaller amount will be eliminated in the urine than if the vitamin C content of the tissues is normal. A variety of methods have been proposed, including varying amounts of the vitamin, administered orally and intravenously, and a test period of varying duration. The intravenous route of administration and a short test period seem preferable. Following the injection (intravenous) of 100 mg. of ascorbic acid, at least 40 mg. (40 per cent) should be excreted in the urine within three hours in normal subjects. Following the intravenous injection of 1000 mg., at least 400 mg. should be excreted in the urine in five hours. The excretion of quantities less than these is interpreted as indicative of subnormal saturation of the tissues with vitamin C.

There is considerable question as to the significance of results obtained by this procedure. It has been suggested that in some cases abnormal findings may be due to greater utilization of ascorbic acid rather than to subsaturation of the tissues with this factor. Moreover, there is no definite proof of a direct relation between a state of apparent "subsaturation" and the disease conditions in which it may be present. Among these are tuberculosis, rheumatic fever, rheumatoid arthritis, osteomyelitis, congestive heart failure, renal and hepatic disease, gastrointestinal disturbances, purpura, metabolic and endocrine disorders, and malignancy. An increase in ascorbic acid excretion in the urine has been observed after ether anesthesia and, in children, after administration of acetylsalicylic acid. It would appear that the requirement of the organism for vitamin C and the excretion of this factor in the urine can be influenced by a great variety of factors, and that some degree of vitamin C deficiency may contribute to the symptomatology of a number of clinical disorders.

Other Procedures. The ascorbic acid content of the cerebrospinal fluid has been found to range from 0.7 to 2.1 mg. per 100 cc. by some observers and from 1.8 to 4 mg. per 100 cc. by others. The concentration in the cerebrospinal fluid appears to vary roughly directly with that in the blood plasma.

Instead of determining the concentration of ascorbic acid in the blood plasma or urine by titration with dichlorphenolindophenol, this blue dye may be injected intradermally and the time required for its decolorization noted. When a wheal of 2 mm. diameter is produced by the intradermal injection of a solution of the dye, complete decolorization of the latter should occur in less than five minutes if the tissues are fully saturated with vitamin C. Adequate vitamin C nutrition is indicated by decolorization in from five to ten minutes and vitamin C deficiency is present if more than ten minutes are required. Although other reducing substances, such as glutathione, may cause decolorization of the dye, this procedure may be useful as a rapid clinical test for the demonstration of probable vitamin C deficiency in cases in which the titration method cannot be employed. Its accuracy is, however, questionable.

Capillary Resistance (Fragility) Test. This is designed to disclose, by the application of mechanical stress, increased fragility of the capillary walls. This is generally ascribed to defective formation of the intercellular capillary cement substance, but increased activity of the hyaluronidase-hyaluronic acid system may be involved. A positive reaction may be obtained before the defect manifests itself spontaneously by petechial or other hemorrhages. The mechanical stress is applied in the form of either negative (cupping) or positive (tourniquet) pressures.

These procedures are of value in the diagnosis of ascorbic acid deficiency, but, unfortunately, capillary fragility may be increased in other conditions. They are useful chiefly as screening procedures, a normal (negative) response constituting evidence against serious ascorbic acid deficiency.

VITAMIN D

The inactive natural precursors of the D vitamins, the corresponding provitamins, are steroids (p. 84). At least ten such substances are known, only two of which have been found in nature: (1) ergosterol (provitamin D_2) and (2) 7-dehydrocholesterol (provitamin D_3), the former in plants, the latter in animals. Transformation from the inactive to the active forms is accomplished physiologically by the ultraviolet rays present in sunlight, or artificially by ultraviolet irradiation, and α, β, γ, and other similar types of radiation. The only D vitamins of importance in human nutrition are activated ergosterol (vitamin D_2; calciferol), and activated 7-dehydrocholesterol (vitamin D_3).

Although plant sterols, including ergosterol, are not absorbed from the gastrointestinal tract, irradiated ergosterol is readily absorbed in the small intestine and also from the skin surface. Being fat-soluble, its absorption from the bowel is enhanced by factors which favor fat absorption, including an adequate quantity of bile salts.

Little is known about the intermediary metabolism of vitamin D. Adequate liver function is apparently necessary for optimal antirachitic activity. This may be related to the fact that the liver is the chief site of storage of vitamin D, although significant amounts are stored also in the skin and brain and small amounts in the lungs, spleen and bones. Administered vitamin D is retained in the body, especially in the liver, with remarkable tenacity, being released very gradually. A small dose exerts an influence for several weeks, a large dose for much longer periods, undergoing very little destruction or excretion under normal conditions. A small amount may be excreted in the bile, but is apparently largely reabsorbed in the intestine. None is eliminated in the urine. The vitamin D content of milk reflects the intake; e.g., ingestion of a single large dose (600,000 I.U.) increases the concentration in the milk (human) from about 10 I.U./liter to 1000 I.U./liter in one day, the level being still elevated (100 I.U./liter) after one month.

The over-all effect of vitamin D is to increase the availability and retention of calcium and phosphorus and their utilization for proper mineralization of the skeleton. Except in a few particulars, the exact manner in which these effects are produced is not known.

1. Vitamin D promotes absorption of calcium from the distal ileum, where absorption is otherwise relatively poor, but not from the upper ileum and jejunum, where calcium is absorbed more readily.

2. Intestinal absorption of phosphate is apparently increased. It is not certain whether this is dependent entirely on increased calcium absorption.

3. Vitamin D is necessary for proper growth, perhaps through its action in promoting endochondral growth of long bones and mineralization of the "zone of provisional calcification" (antirachitic action).

4. It promotes, in some way, mineralization of the skeleton, in both adult and growing organisms, perhaps by providing an adequately balanced supply of available calcium and phosphate in the body fluids.

5. It may aid in mobilizing inorganic phosphate from organic sources in the bones.

6. It lowers the pH in the colon, cecum, and distal ileum, the urinary pH increasing simultaneously. This may be, in part at least, secondary to increased absorption of calcium, decreasing the base of the intestinal contents.

7. It counteracts the inhibitory effect of calcium ions on the hydrolysis of phytate (inositol hexaphosphate). In adequate amounts and in the presence of a high calcium intake, it suppresses the anticalcifying and rachitogenic effect of phytate. The mechanism of action is unknown.

Deficiency Manifestations. Vitamin D deficiency during the period of skeletal growth results in rickets (rachitis), with a characteristic defect in endochondral bone growth and mineralization of the "zone of provisional calcification" of long bones (junction of epiphysis and diaphysis) and corresponding areas of flat bones. This lesion cannot occur in fully grown bones, the characteristic skeletal manifestation of deficiency in adults being a type of defective mineralization of osteoid tissue termed "osteomalacia."

METABOLIC MANIFESTATIONS. Vitamin D deficiency results in decreased net retention of calcium and inorganic phosphate in the organism. The mineral content of the bones is diminished, the water and organic matter being correspondingly increased. The bones have a lower calcium, phosphate, and carbonate content and a higher magnesium content. Urinary excretion of calcium and phosphate falls and fecal excretion increases. The serum inorganic phosphate concentration usually falls, whereas the serum calcium usually remains within normal limits for some time; in advanced deficiency it, too, decreases, often to markedly hypocalcemic levels (infantile tetany). The serum alkaline phosphatase activity increases, usually in proportion to the severity of the skeletal defect.

The sequence of events is believed to be as follows:

1. There is decreased intestinal absorption of calcium, resulting in tendency toward decrease in serum calcium and decrease in urine calcium.

2. As soon as the serum calcium tends to fall the parathyroids are stimulated to increased activity (also hyperplasia), maintaining the serum calcium level, now at the expense of the skeleton (p. 238).

3. Increased parathyroid activity results in increased urinary excretion of inorganic phosphate. This may be due also to a direct effect of vitamin D deficiency on renal tubular absorption of phosphate.

4. Increase in fecal phosphate may result, in part at least, from diminished absorption due to the excess of unabsorbed calcium in the bowel (unfavorable $Ca : PO_4$ ratio).

5. As deficiency progresses, the available mobilizable mineral reserves of the skeleton may become depleted to the extent that the serum calcium level cannot be supported adequately, despite the increased parathyroid activity. Hypocalcemia then develops and, if of sufficient degree, is manifested clinically by neuromuscular hyperexcitability (infantile or osteomalacic tetany).

6. Decreased mineralization of the bones, both generalized and, in growing long bones, also in the zone of provisional calcification, results in softening and

deformities. This strain constitutes a stimulus to proliferation of osteoblasts, which form alkaline phosphatase. The local increase in this enzyme is reflected in increased alkaline phosphatase activity in the blood.

All of the above manifestations are reversed promptly after administration of adequate amounts of vitamin D, calcium, and phosphorus. The increased alkaline phosphatase activity is the last abnormality to disappear.

DEMONSTRATION OF VITAMIN D DEFICIENCY. This depends on the presence of (1) suggestive clinical minifestations, (2) characteristic roentgenographic abnormalities, and (3) metabolic aberrations. The most important of the latter, which are discussed in detail elsewhere, are: (1) hypophosphatemia (p. 243); (2) increased serum alkaline phosphatase activity (p. 250); (3) normal or decreased serum calcium (p. 238); (4) decreased urinary and increased fecal excretion of calcium and phosphate (p. 466). Prompt response to specific therapy, except in cases of so-called "vitamin D-resistant rickets," is of great diagnostic importance.

Effects of Excess Vitamin D. Vitamin D is well tolerated in doses many times the normal requirement. However, seriously deleterious effects may be produced by extremely large amounts (500–1000 times the normal requirement). The early symptoms are due chiefly perhaps to the induced hypercalcemia (increased intestinal absorption of calcium and possibly increased skeletal mobilization). These include anorexia, thirst, lassitude, constipation, and polyuria, followed later by nausea, vomiting and diarrhea. Hyperphosphatemia may occur.

Increased urinary excretion of calcium and phosphate may lead to urinary lithiasis and the hypercalcemia and hyperphosphatemia may lead to metastatic calcification. The kidneys, arteries, bronchi, pulmonary alveoli, muscles, and gastric mucosa are principally involved. Renal failure may develop, leading to death. In growing children there may be excessive mineralization of the zone of provisional calcification at the expense of the diaphysis, which undergoes demineralization (with extremely large doses).

VITAMIN K

This is a fat-soluble substance required for the formation of prothrombin, which is an essential factor in the mechanism of coagulation of blood. The great practical importance of this vitamin arose out of the observation that certain common hemorrhagic disorders in man are dependent upon hypoprothrombinemia (e.g., in obstructive and hepatocellular jaundice; in the newborn; in certain intestinal disorders). Administration of vitamin K was found to be strikingly effective in controlling the hemorrhagic diathesis in many of these conditions.

The several substances, natural and synthetic, with vitamin K (antihemorrhagic) activity are naphthoquinones. It has been suggested that the relative effectiveness of these compounds in this connection may be related to their capacity for forming 2-methyl-1,4-naphthoquinone ("menadione") in the body; this is the most potent known substance exerting this effect.

Two naturally occurring vitamins K have been identified. Vitamin K_1, isolated originally from alfalfa, and vitamin K_2, isolated originally from putrid fish meal. Phthiocol is a constituent of tubercle bacilli possessing slight vitamin K activity. Menadione (2-methyl-1,4-naphthoquinone), because of its high activity, is the most important of the synthetic vitamins K, a number of which have been prepared. Vitamin K_1 has also been synthesized.

The natural vitamins K are "fat-soluble vitamins," i.e., they are insoluble in water and are quite soluble in most fat solvents. The synthetic forms, lacking the long hydrocarbon chain, are somewhat soluble in water, but not sufficiently so for practical purposes. However, the hydroquinones of the vitamins K form esters (disulfate, diphosphate, diacetate) which are more soluble (water), less irritating, and more stable than the parent compounds, although less active. Certain of these are available and satisfactory for parenteral administration, e.g., 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate; 4-amino-2-methyl-1-naphthol hydrochloride.

Dietary supply of vitamin K is of little practical importance under ordinary circumstances, because adequate amounts are synthesized by the normal intestinal bacteria. An exogenous supply may become important, however, in individuals in whom growth of intestinal bacteria is inhibited (by certain antibiotics and sulfonamides) for therapeutic purposes (e.g., preliminary to surgical operations on the gastrointestinal tract).

A normal diet contains an abundance of vitamin K, which is also formed by intestinal bacteria (chiefly Escherichia coli) in amounts adequate to meet normal requirements. Being fat-soluble, its absorption, which occurs predominantly in the jejunum, by way of the lymphatics, is influenced by factors which affect the absorption of lipids. Adequate amounts of bile salts must be present for optimal absorption, a fact of great importance in relation to the hemorrhagic manifestations of biliary obstruction. Absorption is diminished by large amounts of liquid petrolatum.

Vitamin K has not been found consistently in the blood stream in significant amounts. During pregnancy, it apparently passes readily from the mother to the fetus. The capacity of the organism for storing this vitamin is extremely limited, at least in experimental animals, and evidence suggests that this is true also of man. Both vitamin K and prothrombin are utilized or metabolized rather rapidly, so that deficiency manifestations appear after a relatively brief period of deprivation (24 to 48 hours in rats).

Vitamin K is apparently not excreted in the urine or bile. It has been found in the milk of women receiving adequate amounts of the vitamin. Rather large quantities may be present in the feces; whereas this may represent actual excretion by the intestinal mucosa, a more probable source is the intestinal bacterial flora.

Administration of excessive amounts of vitamin A results, in certain species, in hypoprothrombinemia and hemorrhagic manifestations which are prevented by simultaneous administration of vitamin K. It is believed that this effect may be due to interference with bacterial synthesis of vitamin K in the intestine.

Vitamin K is apparently essential for the formation of prothrombin by the hepatic polygonal cells. This is its only known function. The mechanism of its action is unknown. Prothrombin does not possess vitamin K activity, nor does the vitamin enter into the prothrombin molecule. It has been suggested that it acts as a coenzyme in the synthesis of prothrombin or as an oxidation-reduction catalyst in this process.

Deficiency. Vitamin K deficiency can be induced by dietary restriction in several species (chick and other birds, rat, mouse, rabbit). In man, because of adequate synthesis of the vitamin by intestinal bacteria, deficiency rarely results from dietary inadequacy. The low plasma prothrombin which occurs consistently in the newborn, during the first few days of life, is attributed to vitamin K deficiency, due in part to inadequacy of the intestinal bacterial flora, and in part perhaps to inadequate bile flow and intestinal hypermotility, with consequent poor absorption.

Deficiency manifestations result usually from conditions which interfere with (1) absorption of the vitamin from the intestine, (2) its utilization in prothrombin formation, or (3) its production by intestinal bacteria.

(1) IMPAIRED ABSORPTION. Bile acids appear to act as carrying agents for the passage of vitamin K across the intestinal wall, a function similar to that which they perform for vitamin D and carotene. Absorption from the intestine is impaired in the absence of adequate amounts of bile (bile acids) in the intestine, as in obstructive jaundice and external bile fistula, with a consequent fall in plasma prothrombin and development of a hemorrhagic tendency. Adequate absorption under such circumstances follows administration of bile salts, the most effective of which appears to be sodium desoxycholate. Inadequate absorption of vitamin K may occur in conditions of severely disturbed intestinal function, as in patients with intestinal obstruction, intestinal fistula, enterostomy, gastrocolic fistula, chronic pancreatitis, sprue, celiac disease, chronic ulcerative colitis, prolonged diarrhea, and anorexia nervosa. Absorption is also interfered with by large amounts of liquid petrolatum.

(2) HEPATIC DISEASE. The liver is the chief if not the only site of formation of plasma prothrombin, and an adequately functioning liver is essential for the proper utilization of vitamin K in this process. The plasma prothrombin may be diminished experimentally by partial hepatectomy and administration of hepatotoxic agents, such as chloroform, carbon tetrachloride, and phosphorus. The same phenomenon occurs in many patients with hepatocellular damage due to any cause (p. 511). In contrast to the beneficial effects of vitamin K administration in patients with uncomplicated obstructive jaundice, those with extensive hepatocellular damage characteristically exhibit little or no elevation of prothrombin following injection of vitamin K. This failure to respond to this agent has been suggested as a test of liver function (p. 512).

(3) MISCELLANEOUS. The plasma prothrombin is low in the newborn infant (physiological hypoprothrombinemia) and is one cause of hemorrhagic disease of the newborn. A condition of idiopathic hypoprothrombinemia in adults has been described. Large doses of salicylates and barbiturates may be followed by decrease in plasma prothrombin. Hypoprothrombinemia has been observed in pulmonary

tuberculosis, pneumonia, following hemorrhage, and after operations, particularly on the biliary tract. In the majority of these conditions, if not accompanied by severe hepatic damage, the prothrombin level increases after administration of adequate doses of vitamin K. Extremely large doses are required to combat the hypoprothrombinemia induced by administration of dicoumarin and heparin. Hypoprothrombinemia may be induced also by oral administration of certain sulfonamides and antibiotics for the purpose of inhibiting growth of intestinal bacteria, e.g., prior to operations on the gastrointestinal tract. This can be overcome by adequate vitamin K therapy.

Demonstration of Vitamin K Deficiency. The methods employed for the demonstration of vitamin K deficiency consist in determination of the quantity of prothrombin in the plasma. These methods are indirect in nature. The one-stage method (Quick) commonly employed consists in the determination of the clotting time of oxalated plasma at 37.5° C. after addition of an excess of thromboplastin and a fixed amount of calcium. The two-stage method is more satisfactory but more troublesome. In the first, or prothrombin conversion stage, prothrombin is converted completely to thrombin with an optimal amount of calcium and an excess of thromboplastin. In the second, or clotting stage, the amount of thrombin formed is estimated by the time required for clotting of a standard fibrinogen solution.

Decrease in prothrombin is evidenced by prolongation of the clotting time (''prothrombin time'') under these circumstances (see p. 511). Experience has shown that bleeding occurs commonly in patients with obstructive jaundice, bile fistula, and other conditions mentioned above, when the plasma prothrombin concentration, as measured by these tests, falls below 30 to 40 per cent of normal. Values of 40 to 70 per cent of normal are potentially dangerous.

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CHAPTER XIX

Chemical Investigation of Gastric Function

Chemical examination of the gastric contents may yield valuable information regarding both the secretory and the motor activities of the stomach, and may reveal the presence of abnormal substances indicative of pathologic conditions. Microscopical study constitutes an essential part of the examination of the gastric contents but must necessarily be omitted from a discussion of the chemical aspects of functional diagnosis. In order to obtain complete evidence regarding the state of gastric functional activity, the contents of the stomach must be examined both during the interdigestive period (fasting stomach) and during the period of digestion, or stimulation.

The most important specific secretory products of gastric activity are (1) hydrochloric acid, (2) enzymes (pepsin and a weak lipase) and (3) mucus. Gastric juice contains also amino acids, histamine and other amines, urea, ammonia and neutral inorganic salts, such as chlorides. The true gastric glands, i.e., those which secrete HCl and enzymes, occur in the body and fundus, while the glands of the pylorus and cardia secrete mucus but probably no HCl and little or no pepsin. There are three types of cell in the true gastric glands: (*a*) parietal or border cells, which secrete HCl at a rather constant concentration of about 0.155 normal; (*b*) chief cells of the body of the glands, which secrete pepsin; (*c*) chief cells of the neck of the glands, which secrete mucus ("dissolved mucus").

Gastric secretion is commonly described as consisting of three phases, (1) a psychic or cephalic phase, (2) a gastric phase and (3) an intestinal phase.

Psychic or Cephalic Phase. This is due to a nervous mechanism mediated through the vagus. It is evoked by pleasurable sensations accompanying the thought, sight, smell and taste of palatable food, and has been elicited by hypnotic suggestion. Inhibition of secretion may result from psychic influences such as worry and anxiety, and the sight or smell of disagreeable food.

Gastric Phase. Chemical stimulation of the gastric mucosa in the pyloric antrum causes the release of a substance, gastrin (gastric hormone), which, transmitted by the blood stream, acts as a gastric secretagogue. There is evidence that a local nervous mechanism (vagus terminals in the stomach wall) is involved in the production or liberation of this hormone.

Intestinal Phase. Certain products of gastric digestion, when they enter the duodenum, act as chemical excitants to gastric secretion. This is true also of water, meat extracts, albumoses, peptones, saponin, soaps and magnesium sulfate. Although a substance ("gastric secretin") which stimulates gastric secretion has been extracted from the intestinal mucosa, it is generally believed that the intestinal phase is due to secretagogues in the food, absorbed from the intestine, the vagus terminals in the wall of the stomach or intestine being involved in the mechanism.

The intestinal phase of gastric secretion is inhibited by fat (before absorption), the acidity, volume and especially peptic activity being lowered. A similar effect is produced by injection of extracts of intestinal mucosa or urine; the factors responsible for this effect have been termed enterogastrone and urogastrone, respectively.

Other Factors. Insulin (hypoglycemia) stimulates gastric secretion through the vagus mechanism, the volume, acidity and peptic activity of the gastric juice being increased. Mecholyl, pilocarpine, and nicotine increase the volume considerably and the acidity somewhat. Alcohol is a powerful stimulant (acidity and volume), while acids and atropine are secretory depressants.

Different foodstuffs influence the secretion in different ways. For example, meat produces a juice of high acid and moderate pepsin content, bread a juice of low acid and high pepsin content, and milk a juice of moderate acid and low pepsin content. Fat depresses peptic activity relatively more than acidity or volume.

NORMAL GASTRIC JUICE

Gastric juice is secreted continually. The acidity of the parietal cell secretion is rather constant (about 0.155 normal), and is dependent upon the electrolyte composition of the blood plasma. Variations in acidity of the mixed gastric juice (normal and abnormal) are due, therefore, not to variations in the concentration at which HCl is secreted, but (*a*) to variations in the relative proportions of the different gastric secretions (e.g., by mucous cells, chief cells, parietal cells), and (*b*) to such neutralizing substances as saliva, food, mucus, and regurgitated materials (e.g., pancreatic juice, bile, duodenal secretion) from the duodenum.

Gastric Residuum. Valuable information may be obtained by the examination of the stomach contents during the interdigestive period (gastric residuum). The following chemical and gross physical characteristics are of importance from the standpoint of functional diagnosis.

Amount. The quantity of material normally found in the fasting stomach varies from 20 to 100 cc., averaging about 50 cc. An increase in the volume of the gastric residuum may be due to hypersecretion, retention or regurgitation from the duodenum. These conditions may be differentiated on the basis of other observations such as the presence of food particles in retention, the presence of abnormal quantities of bile and duodenal enzymes in regurgitation, and the absence of these factors in true hypersecretion, in which condition the gastric contents, apart from their increased volume and perhaps increased acidity, may be essentially normal.

Color. Freshly secreted gastric juice is colorless. However, in about 55 per cent of normal individuals the gastric residuum is either yellow or green, due to regurgitation of bile from the duodenum, which occurs in about 25 per cent of normal individuals, or to the presence of molds or the Cryptococcus salmonicus. Increased quantities of bile in the stomach, when not due to retching incident to the passage of the tube, result from intestinal obstruction or ileal stasis. A bright or dark red, brown or black color in the residuum is usually indicative of the presence of blood (see below).

Consistency. The normal gastric residuum is rather fluid in consistency, containing no solid food particles and only a small quantity of ropy mucus which may be derived from the nasopharnyx. The presence of an increased quantity of sediment is usually indicative of retention, and increased quantities of mucus are found in catarrhal inflammations of the stomach.

Blood. The benzidine test is the one most commonly employed for the detection of blood in the gastric contents. A trace of bright red, aerated blood in specimens extracted through the ordinary metal-tipped tube is most commonly due to accidental trauma to the gastric mucous membrane. The incidence of accidental blood may be diminished by the use of a paraffin or rubber-coated tip. Pathologically, the gross appearance of specimens containing blood depends upon the extent of the hemorrhage, the length of time the blood has remained in the stomach and the degree of acidity of the gastric contents. In the presence of hydrochloric acid the red cells are hemolyzed, the hemoglobin being converted to acid hematin, which is dark brown in color. Blood which has resided in the stomach for relatively long periods of time is therefore usually dark and well mixed with the gastric contents. If coagulation has occurred, as is frequently the case in carcinoma, the clot may be partially disintegrated, having the so-called characteristic "coffee-grounds" appearance. The presence of blood in the gastric residuum may be due to such lesions as carcinoma of the stomach, portal cirrhosis, chronic passive congestion of the stomach, peptic ulcer, gastric lues, acute gastritis, and hemorrhagic blood dyscrasias, including purpura hemorrhagica, acute leukemia, agranulocytosis, aplastic anemia, and so on. It must be remembered that blood derived from the gums and from nasopharyngeal, laryngeal, tracheobronchial and pulmonary lesions may be swallowed.

Organic Acids. Lactic, butyric and other fatty acids may be found in the gastric contents. Some observers believe that lactic acid is secreted by the gastric mucosa in carcinoma of the stomach; however, the consensus is that organic acids result from stagnation of the gastric contents with consequent bacterial action and fermentation. Since these processes are inhibited by high free acidity, lactic acid is most commonly found in association with gastric retention and hypochlorhydria, the combination of the three being rather significant of carcinoma of the stomach.

Enzymes. The normal gastric residuum contains pepsin and rennin (?). Trypsin is frequently found to be present as a result of regurgitation from the duodenum. The absence of pepsin, occurring in conjunction with achlorhydria, constitutes true achylia, which is encountered typically in pernicious anemia and in subacute combined degeneration of the spinal cord. This enzyme is practically never absent if free hydrochloric acid is present in the gastric secretion.

Free and Total Acidity. Gastric acidity is usually expressed in terms of the number of cubic centimeters of tenth-normal sodium hydroxide required to neutralize 100 cc. of gastric contents ("clinical units," or mEq./liter). The free HCl content of the residuum normally ranges from 0 to 30, with an average value of 18.5 (cc. N/10 NaOH to neutralize 100 cc.). This is at times expressed in terms of grams of hydrochloric acid per 100 cc. of gastric contents, ranging from 0 to 0.1095 gm. (or 0–30 mEq./liter), with an average of 0.0675 gm. The total acidity includes acidity due to free hydrochloric acid, hydrochloric acid combined with protein, acid salts (phosphates), and organic acids such as lactic and butyric acids (abnormal). The total acidity of the normal residuum ranges from 10 to 50 units or mEq./liter, averaging 30. In terms of grams HCl per 100 cc., the total acidity ranges from 0.0365 to 0.1825 gm., with an average value of 0.1095 gm.

A condition of hypoacidity cannot be diagnosed by examination of the gastric residuum since free hydrochloric acid may be normally absent and the total acidity extremely low. Free HCl is practically never found when the total acidity is below 10. The absence of both free HCl and total acidity, particularly if pepsin is also lacking, may however be of significance. Free HCl values above 30 or total acid values above 50 should be regarded as abnormally high, constituting a state of hyperacidity.

INVESTIGATION OF GASTRIC SECRETORY ACTIVITY

Many procedures have been employed for the evaluation of gastric secretory activity. They have been designed chiefly to aid in the diagnosis of peptic ulcer (gastric and duodenal), carcinoma of the stomach, and pernicious anemia (achlorhydria). Their usefulness is based upon the observations (*a*) that hypersecretion, i.e., hyperacidity, occurs commonly in duodenal ulcer, (*b*) that hyposecretion, i.e., hypoacidity, is a common feature of gastric cancer, and (*c*) that pernicious anemia is virtually invariably accompanied by achlorhydria.

The presence of hypersecretion may at times be manifested by an abnormally high concentration of free HCl or total Cl in the gastric residuum (p. 479 ff.). However, this is not invariably the case, and normal values for HCl in the residuum do not eliminate the possibility of a hypersecretory response to stimulation. Moreover, absence of free HCl in the residuum is by no means always indicative of achlorhydria or hyposecretion. For these reasons, procedures other than the mere examination of a sample of the fasting gastric contents must usually be employed for purposes of differential diagnosis.

Basal Gastric Secretion. Normal adults secrete an average of about 2500 cc. of gastric juice daily. The volume of nocturnal secretion (6 P.M. to 6 A.M., following mild stimulation) ranges from about 140 cc. to about 1200 cc., the maximum free acidity from 1 to 90 units (av. 30) (or mEq. HCl/liter), and the total free HCl secreted 10 to 3500 mg. (av. 660). The most important factor in differential diagnosis is probably the total amount of HCl secreted. Of particular significance, in view of the wide normal variation, is the fact that 85 per cent of normal subjects secrete less than 1000 mg. HCl during the 12-hour nocturnal period, 50 per cent less than 500 mg., and 30 per cent less than 200 mg.

In sharp contrast, the average volume in subjects with duodenal ulcer is about double that in normal individuals. The free acidity ranges from 10 to 115 (av. 60) units, and the total HCl secreted from 400 to 6000 mg. (av. 2300), being over 2000 mg. in 50 per cent of cases and over 1000 mg. in 80 per cent.

Low volumes are obtained in benign gastric ulcer and particularly in gastric cancer. In gastric ulcer, the average free HCl secretion is somewhat lower than in normal subjects, viz., about 450 mg., being less than 500 mg. in 65 per cent of cases. This is perhaps due to associated atrophic gastritis. Lower values, averaging 250 mg., are obtained in cancer of the stomach, being less than 500 mg. in 80 per cent of cases.

The chief disadvantage of the above procedure is the prolonged nocturnal period of collection of gastric juice. Comparable results have been obtained with briefer periods of study during the morning, e.g., 10 or 15 minute aspirations for one hour. The maximum HCl values obtained by this method differ in the conditions referred to above in much the same manner as do findings with the 12-hour nocturnal collections. It has been found, for example, that whereas about 85 per cent of normal subjects secrete a maximum of less than 0.8 mEq. HCl per 10-minute period, this value is exceeded in 80 per cent of cases of duodenal ulcer. Moreover, anacidity occurs during one or more of these 10- or 15-minute periods in many normal subjects, but only rarely in patients with duodenal ulcer.

Test Meals. In former years, a number of different test meals were employed to study the secretory (and motor) activity of the stomach. These are not used extensively at the present time.

The Ewald meal consists of either 1 roll or 2 slices of unbuttered bread or toast (about 35 gm.) and 8 ounces of water or weak tea (no sugar or cream). This serves as a mild stimulant of gastric secretion. The Riegel meal consists of 200 cc. of beef broth, 150 to 200 gm. of broiled beefsteak, and 100 gm. of mashed potatoes. This provides a more potent stimulus than does the carbohydrate Ewald meal, and was formerly used in cases of suspected hypochlorhydria or achlorhydria. It has been largely replaced by the alcohol, caffeine, and histamine procedures.

The diagnostic value of the Ewald meal and subsequent fractional gastric analysis is questionable, in view of the availability of other procedures. However, it seems advisable to outline the usual findings in normal individuals.

Three types of normal response may be obtained with the Ewald meal (Fig. 46).

1. *Isosecretory*. In about 40 per cent of cases, free HCl, if not present in the residuum, usually appears in the first sample (15 minutes). It increases gradually to a maximum of 40 to 60 units in 60 to 75 minutes, then falls gradually to 10 to 15 units in two to two and one-half hours. Free HCl is almost never present with total acidity values below 10 units, and is practically never absent if this value exceeds 13.5. The curve of total acidity parallels that of free HCl, usually attaining a maximum of 60 to 70 units.

2. *Hypersecretory*. In about 30 per cent of cases free HCl, usually present in the residuum, rises rather rapidly to a maximum of 70 to 90 units in about two hours, and then falls rather slowly.

3. *Hyposecretory.* In about 30 per cent of cases free HCl is absent in the residuum and increases very slowly to a maximum of 30 to 40 units.

It is apparent that normal individuals exhibit an extremely wide variation in their response to the Ewald meal. The values tend to be somewhat lower in children than in adults. Maximum acidity tends to fall after thirty years of age in men and after fifty years in women.

Alcohol Stimulation. After collection of basal secretion (15–60 minutes), 50 cc. of 7 per cent ethyl alcohol are instilled into the stomach through the tube. Samples are aspirated for analysis at intervals (10–20 minutes) for 1½ to 2 hours. This procedure possesses the advantages of ease of withdrawal of samples, ease of examination of specimens, adequate stimulation of secretion, and the possibility of a more exact measurement of the volume of secretion than is possible with test meals.

Caffeine Stimulation. After a basal collection period (10–15 minute samples for 30–60 minutes), 500 mg. caffeine sodium benzoate in 200 cc. of warm water are instilled into the stomach. The entire stomach contents are aspirated after 30 minutes. Samples are withdrawn at intervals of 10 minutes for 90 minutes or until the basal level is reached. Measurements are made of volume and acidity in all samples.

In normal subjects, secretion reaches a maximum usually at about 30 minutes, and returns to the basal level at 60 to 80 minutes. The total HCl secretion is less than 300 mg. in over 90 per cent of normal individuals and in less than 10 per cent of subjects with duodenal ulcer, who also exhibit a prolonged curve of secretion.

Histamine Stimulation. Histamine is a powerful gastric secretory stimulant, producing a juice of maximal acidity and relatively low pepsin content. After a basal collection period (15-minute samples for 30–60 minutes), histamine base is injected subcutaneously in a dosage of 0.01 mg./kg. body weight. Some use a standard total dose of 0.3 cc. of a 1 : 1000 solution of histamine phosphate. The stomach is then emptied at intervals of 15 minutes for 60 to 90 minutes, or until the basal level (volume and acidity) is reached.

In normal subjects, the free HCl increases rather promptly, reaching a maximum usually in 20 to 40 minutes, then falling. The volume of gastric juice varies correspondingly. The maximum response in normal subjects, excluding those with achlorhydria, may range from 30 to 160 units (30–160 millimols HCl or 0.1–0.6% HCl). Because it produces a maximal acid response, this procedure must be employed before a diagnosis of achlorhydria can be established with certainty.

A double histamine test has been proposed for demonstrating the capacity of the stomach for maintaining a maximal secretion over prolonged periods of time. The second injection is given sixty to ninety minutes after the first, when the initial curve is beginning to fall. In normal subjects a second rise and fall occur, similar to the first curve in shape and magnitude. In subjects with hypersecretion (e.g., duodenal ulcer), the peak of acidity reached after the first stimulation is maintained at a relatively constant level during the double test period.

The histamine test should not be performed in subjects with a history of allergy. Epinephrine and an injectable antihistaminic agent should be at hand for administration in the event of an untoward reaction.

Insulin (Hypoglycemia) Stimulation. Hypoglycemia causes stimulation of the vagus centers in the brain, one manifestation of which is increased gastric secretion. This procedure is used chiefly to ascertain the effectiveness of vagotomy in patients with ulcer.

After a basal collection period (15 minute samples for 45–60 minutes), blood is drawn (for sugar determination) and 20 units of regular insulin are injected intravenously. The stomach is emptied at 15-minute intervals for two hours, and blood is drawn at 30 to 45 minutes (or when hypoglycemic symptoms appear, e.g., salivation, perspiration) and again at the end of the test period. Saliva must be expectorated. Glucose for intravenous administration should be at hand, and sweetened orange juice should be given at the conclusion of the test.

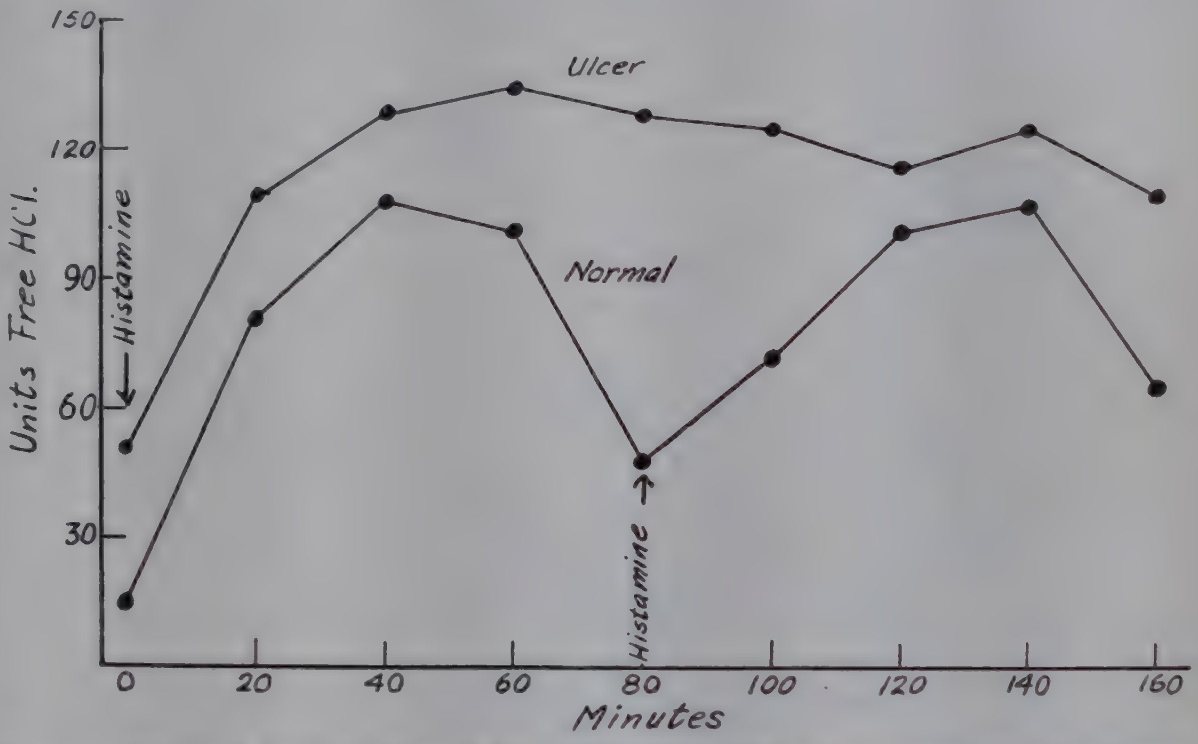

Fig. 45. Curve of concentration of free HCl produced by two consecutive injections of histamine in a normal subject and in a patient with duodenal ulcer.

A positive response, i.e., indicating vagal innervation of the stomach, consists in a rise in free and total acidity above the basal levels. The free HCl may increase to 80 to 90 units (mEq./liter). A negative response is not significant if the blood sugar has not fallen to at least 40 mg./100 cc.

Abnormal Response

From the standpoint of acidity, three main types of response may be obtained under pathologic conditions.

Achlorhydria. The term "achlorhydria" is applied to the absence of free hydrochloric acid in all samples obtained during the digestive period. In some cases the total acidity may rise to 20 to 30, the curve being practically horizontal.

The term "true achlorhydria" is applied to the absence of free HCl from freshly secreted gastric juice. The term "false achlorhydria" is applied to the absence of free HCl from the withdrawn samples due to the fact that it has been combined with and neutralized by the ingested material, duodenal regurgitation, saliva or mucous secretion of the stomach. The two conditions may be differentiated by the determination of the total chloride concentration of the gastric contents, which affords a more accurate index of the actual amount of secreted hydrochloric acid, being normal in false achlorhydria and low in true achlorhydria. True achlorhydria is most commonly observed in pernicious anemia, gastric carcinoma, chronic gastritis, gastric neuroses, oral sepsis, subacute combined degeneration of the cord and severe anemias. Achlorhydria is also encountered occasionally in patients with hyperthyroidism, adrenal insufficiency, diabetes mellitus, pulmonary tuberculosis and generalized arteriosclerosis. The occurrence of achlorhydria in the presence of an ulcerated gastric lesion should arouse suspicion of carcinoma of the stomach. It is present in less than 6 per cent of healthy young adults (twenty to forty years), but increases with advancing age, having been found in 20 to 30 per cent of patients without gastric disease between the ages of sixty and seventy years.

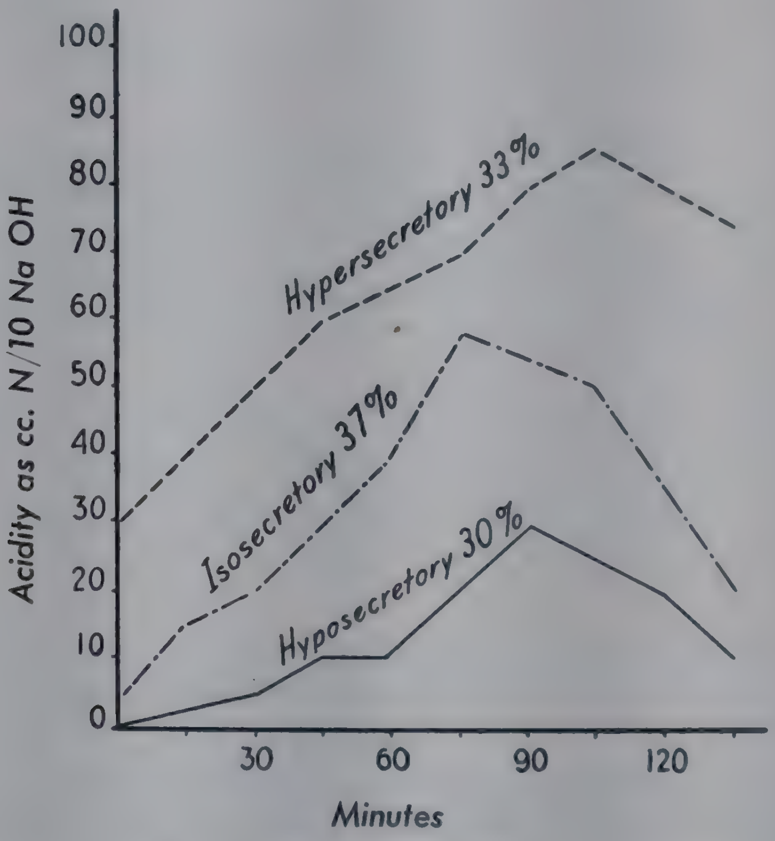

Fig. 46. Normal free HCl curves.

The existence of achlorhydria can be determined with certainty only by histamine stimulation. This produces a maximal acid response, ranging from 30 to 160 clinical units in normal subjects (excluding achlorhydria), with minute volumes of gastric juice of 15 to 60 cc. (average 30 cc.).

There is in some individuals a condition of primary achlorhydria, associated with none of the recognized causes for the absence of free HCl, occurring probably on the basis of some constitutional defect, and showing a definite hereditary and familial tendency. Many of these are instances of true achylia, gastric enzymes also being absent. This condition is of particular interest because of the fact that such individuals appear to be predisposed to the development of pernicious anemia.

Hypoacidity. The term "hypoacidity" (hyposecretion) is applied to acid curves which are lower than that known to be normal for any individual or, in any case, lower than that characterized by the hyposecretory type of normal response. Hypoacidity is commonly observed in carcinoma of the stomach, chronic gastritis, chronic constipation, chronic appendicitis, gastric and other neuroses, mucous colitis, secondary anemia, chronic debilitating disease, particularly tuberculosis, in hyperthyroidism, most cases of gastric, and rarely in duodenal ulcer, and in about 20 per cent of normal individuals, some of whom perhaps belong more properly in the group of neurotics. Subnormal secretion of free HCl and pepsin has been observed in about 75 per cent of normal pregnant women. This observation may be of significance in relation to the development of both iron deficiency anemia (defective iron absorption) and the so-called "pernicious anemia of pregnancy."

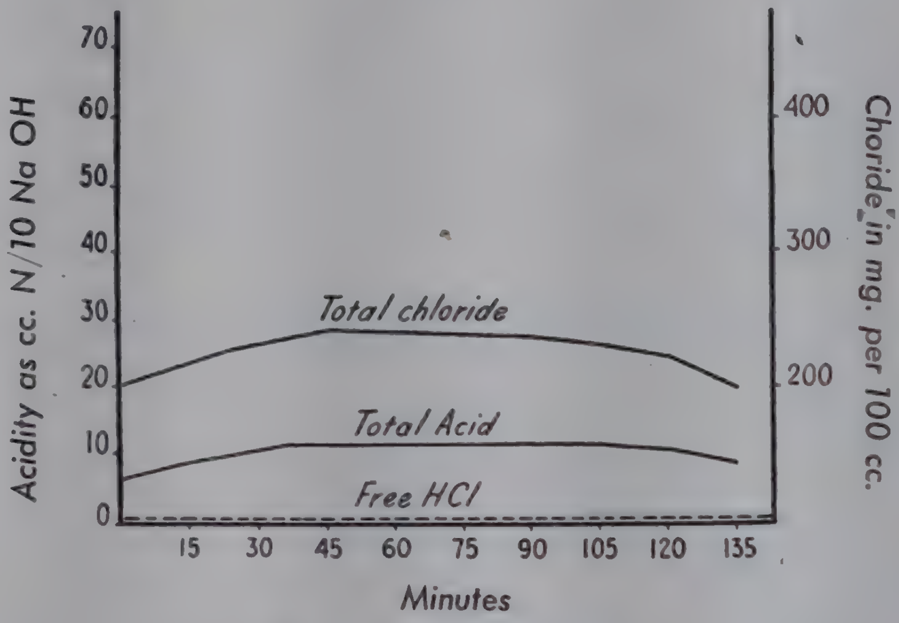

Fig. 47. True achlorhydria.

Hyperacidity (Hypersecretion). A small proportion of normal individuals exhibit high acid values (100–120 total acidity) during the digestive period. More emphasis must be placed upon the interdigestive acidity, the type of acid curve and the rate of gastric evacuation than upon the actual height to which the acidity rises during the digestive stage. Hyperacidity does not imply that the gastric glands are secreting a juice of abnormally high acidity. There is no evidence that the normal maximum of 0.5 to 0.6 per cent HCl (160 clinical units) is ever exceeded. Clinical hyperacidity is due to one or more of the following factors: (*a*) secretion of an abnormally large volume of gastric juice (hypersecretion), (*b*) failure of the volume of secretion to diminish during the second hour and (*c*) delayed gastric evacuation. Rehfuss has classified the various types of hyperacid curves as follows:

(*a*) Larval Hyperacidity. There is a sharp rise in acidity within the first hour of digestion, the remainder of the curve being normal and the residual acidity being within normal limits. Larval hyperacidity may be due to excessive gastric secretion. It is commonly observed in certain gastric neuroses and occasionally in duodenal ulcer. Little practical significance can be attached to this type of acid reaction.

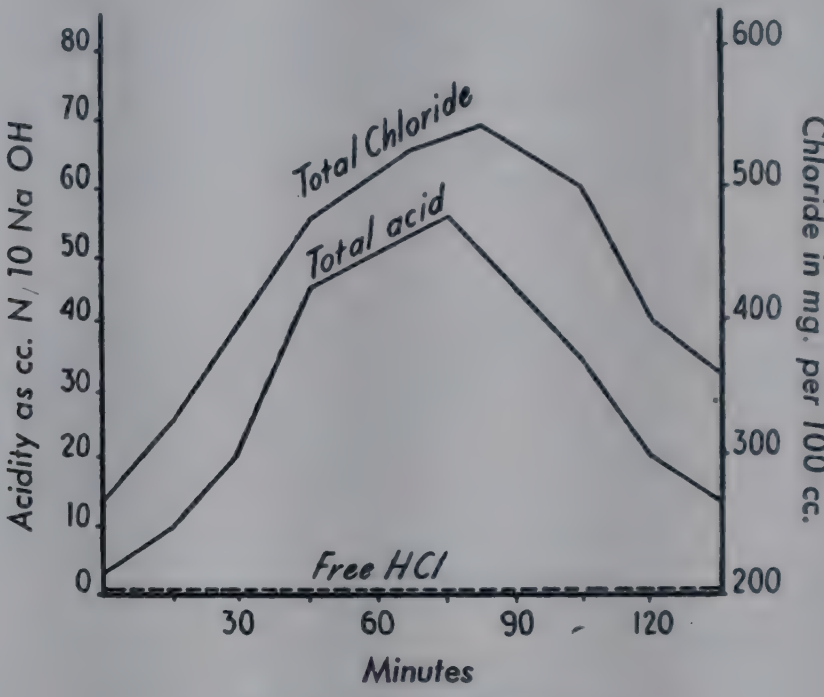

Fig. 48. False achlorhydria.

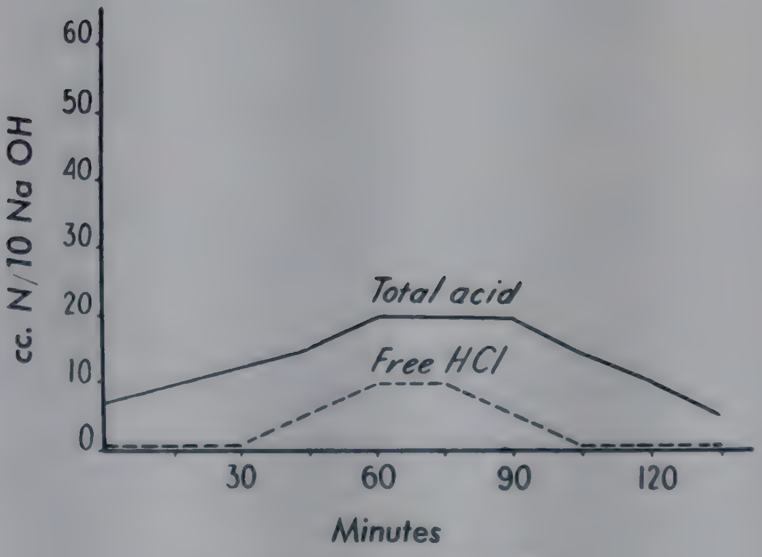

Fig. 49. Hypochlorhydria.

(*b*) Digestive Hyperacidity. This type of reaction resembles the normal hypersecretory curve but may be greatly exaggerated and prolonged. The maximum acidity coincides with the height of the digestive period. Digestive

Table 35. Frequency Distribution of Gastric Acidity in Normal Subjects and in Cases of Duodenal Ulcer and Gastric Carcinoma

	TOTAL ACID UNITS							
	0–19	20–39	40–59	60–79	80–99	100–119	120–139	140–159
	%	%	%	%	%	%	%	%
Normal	7	50	40	3	0	0	0	0
Ulcer	2	9	31	37	19	2	0	0
Gastric carcinoma	85	13	2	0	0	0	0	0
After histamine								
Normal	11	5	8	14	16	19	21	6
Ulcer	0	0	1	2	18	33	36	10
Gastric carcinoma	70	18	7	3	2	0	0	0

hyperacidity is observed in cases of gastric neurosis (vagotonia) and in some patients with duodenal ulcer and cholecystitis.

(*c*) Postdigestive Hyperacidity. The gastric acidity increases steadily throughout the entire digestive period, reaching a maximum in the latter portion of the cycle and being maintained into the interdigestive period. This type of curve is rather characteristic of hyperirritability and pylorospasm but is sometimes due to hypersecretion. It is found in from 70 to 80 per cent of patients with duodenal ulcer, in many individuals with cholecystitis, in cases of chronic appendicitis and chronic constipation, and occasionally in gastric ulcer.

(*d*) Interdigestive Hyperacidity. This type of response may be observed as an independent phenomenon or merely as a prolongation of the postdigestive type of hyperacidity. It is observed most frequently in patients with duodenal ulcer or with chronic cholecystitis and duodenal adhesions but may also be obtained in individuals with gastric neurosis (vagotonia) and in normal individuals who use tobacco to excess.

(*e*) Plateau Curve. In this type of response, which is merely an exaggeration of interdigestive hyperacidity, the entire interdigestive portion of the curve is obliterated. The acidity may fall immediately following the ingestion of a meal, due to dilution of the gastric juice. This preliminary fall is rapidly followed by a sharp rise, the acidity being maintained at a high level for several hours. A plateau curve is characteristic of pyloric stenosis of an advanced degree associated with active gastric secretory activity (stenosing duodenal ulcer).

"Tubeless Gastric Analysis." This procedure was introduced for the purpose of demonstrating gastric HCl secretion without intubation of the stomach. It involves oral administration of a quininium cation exchange resin (2 gm.) which, in the presence of free HCl, releases quinine in exchange for the H ion of the acid. The quinine is absorbed in the intestine and is excreted (about one-third) in the urine, where it can be determined quantitatively. Usually, either caffeine sodium benzoate or histamine is employed as a stimulant to gastric secretion. Urine is usually collected for two hours following ingestion of the resin.

The consensus seems to be that this procedure is useful as a screening test for the detection of achlorhydria, a positive response (i.e., quinine excretion) eliminating the necessity for further study. However a negative response is not absolutely indicative of achlorhydria. Intubation and histamine stimulation are required to establish this diagnosis. This procedure is not satisfactory for the demonstration of hyper- or hyposecretion.

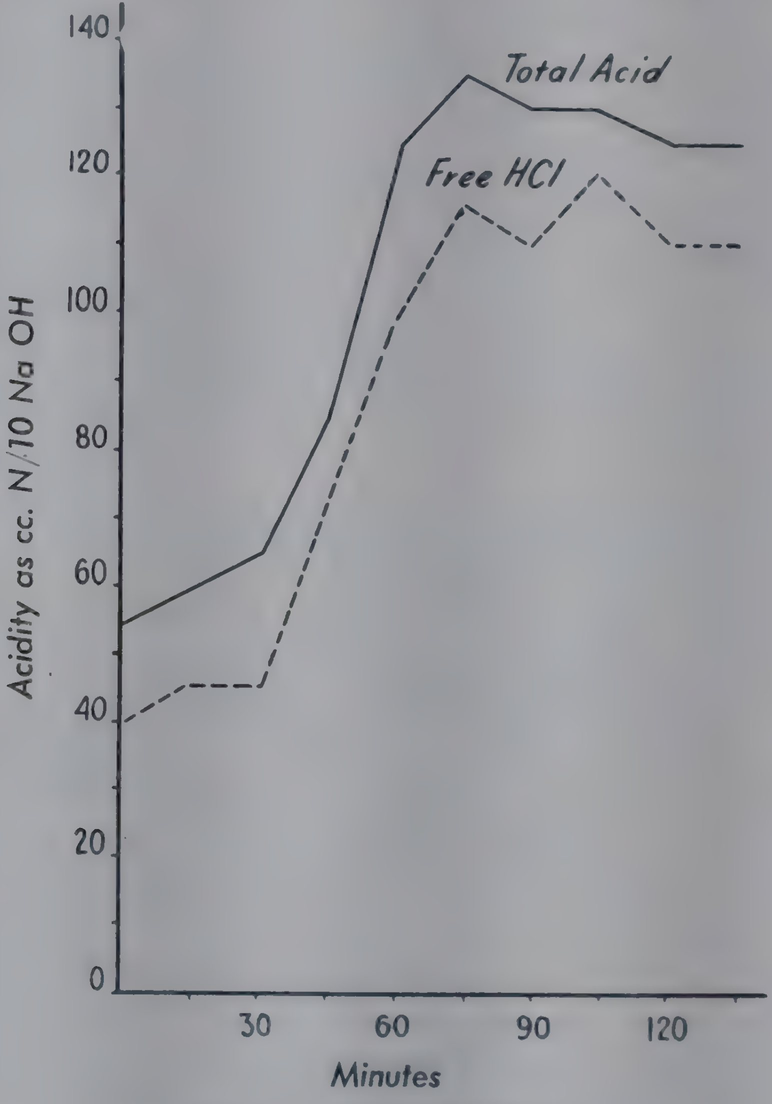

Fig. 50. Hyperchlorhydria.

Peptic Activity

The normal gastric juice contains pepsin. Trypsin may also be present in the gastric contents as a result of regurgitation from the duodenum. Pepsin is never absent from the gastric juice if free hydrochloric acid is present. However, in the absence of free hydrochloric acid, the determination of pepsin is of importance in distinguishing between achlorhydria and achylia.

Achylia is the term applied to the absence of both free hydrochloric acid and gastric enzymes, the total acidity ranging from 0 to 15. True achylia may occur in pernicious anemia, subacute combined degeneration of the cord, severe secondary anemia and following gastro-enteritis in children. Occasionally, free hydrochloric acid and pepsin may be absent from specimens withdrawn following the administration of a test meal, but will be found following the production of maximum stimulation by means of histamine injected subcutaneously. The latter procedure is of value in distinguishing between true and false achylia. True achylia is a very significant feature of pernicious anemia from the standpoint of laboratory diagnosis.

A proteolytic enzyme, apparently pepsin (pepsinogen), is excreted normally in the urine, and has been termed "uropepsin." It apparently enters the blood stream directly from the gastric cells. It has been suggested that measurement of uropepsin excretion might be employed as a substitute for gastric analysis by intubation. Despite occasional satisfactory results, this procedure does not appear to afford a reliable index of gastric secretion in duodenal ulcer, gastric ulcer, and gastric carcinoma. There is a wide range of normal variation, apparently influenced by adrenocortical activity, i.e., increased uropepsin excretion in stress.

Total Chloride

Several factors may operate under normal and abnormal circumstances to modify the state in which HCl exists in the stomach. A portion may remain as secreted in the free state, a portion may combine with proteins present in the stomach, and a variable portion may exist in inorganic combination. In the event of excessive regurgitation of duodenal contents into the stomach or excessive alkaline secretion of the pyloric glands, a large proportion of the secreted hydrochloric acid may be neutralized, and under such circumstances neither the curve of free HCl nor that of total acidity can be regarded as indicating the true state of gastric secretory activity. The curve of total chloride is more nearly representative of the true state of gastric secretory activity since it includes, in addition to free hydrochloric acid and HCl combined with protein, salts of hydrochloric acid which are neutral in reaction. It has frequently been observed that the total chloride curve may continue to rise for a variable period after the acid curve has begun to fall, indicating that HCl is still being secreted but is being neutralized. The determination of total chloride in the gastric contents is particularly valuable in the differentiation between true and false achlorhydria; in true achlorhydria no free HCl appears in the gastric contents during the digestive cycle and the curve of total chloride is correspondingly low; in false achlorhydria a normal amount of HCl may be secreted but is not present in the free state in the gastric contents because of excessive neutralization, and under such circumstances the curve of total chloride may be essentially normal (see Figs. 47, 48).

The total chloride content of the fasting juice of normal subjects ranges from 160 to 550 mg. per 100 cc., or 45 to 155 milliequivalents per liter. With histamine stimulation it may reach a maximum of about 600 mg. per 100 cc., or about 172 mEq. per liter. The total chloride varies within narrower limits than the free acidity.

Bile

Bile pigment may be observed in the gastric residuum in from 25 to 55 per cent of normal individuals. It is particularly frequently observed if violent retching has occurred during the passage of the tube. It is believed by some observers that regurgitation from the duodenum constitutes one of the normal mechanisms for the neutralization of gastric acidity, and, as such, occurs particularly in the later stages of the digestive cycle but may occur to a certain extent throughout the entire period. However, the presence of large amounts of bile in all of the specimens extracted during the digestive phase of gastric activity should be considered abnormal, being usually dependent upon relatively increased intraduodenal tension with a patent pylorus.

Vomitus

Vomitus should be subjected to the same routine examination as the gastric residuum. Vomitus obtained during the digestive period should partake of the characteristics of test specimens removed following the ingestion of a stimulating meal, that obtained during the interdigestive phase constituting essentially the fasting contents of the stomach. The observations made regarding the significance of various findings in the aspirated gastric contents apply equally to the vomitus.

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CHAPTER XX

Pancreatic Function

The metabolic significance of the pancreas lies in its internal and external secretions. The internal secretion, insulin, is produced in the beta-cells of the islands of Langerhans, absorbed directly into the blood and plays an important part in the metabolism of carbohydrates, as is indicated in the consideration of carbohydrate metabolism (p. 14) and diabetes mellitus (p. 67 ff.). There is evidence that a hyperglycemic factor, glucagon (p. 17), is secreted by the alpha-cells of the islets. The external secretion, produced by the acinous cells of the pancreas, is strongly alkaline in reaction due to the presence of bicarbonate, and contains a small amount of coagulable protein and inorganic constituents, the most important constituents, however, from a functional standpoint, being three enzymes or their zymogens, namely, trypsin, a proteolytic enzyme, pancreatic amylase, an amylolytic enzyme, and lipase, a lipolytic enzyme. The determination of pancreatic functional efficiency will be discussed from the standpoint of its external secretion, since its internal secretory function is affected in a relatively small proportion of cases of pancreatic disease (about 35 per cent), and is considered in detail elsewhere (p. 67 ff.).

Pancreatic acinar secretion is under hormonal and nervous control. *Secretin* is the term applied to a pancreatic secretagogue obtained by extraction of the upper intestinal mucosa. Entering the blood stream, it exerts a rather specific effect upon the acinar cells, producing an increased volume of fluid of high alkalinity, but the concentration of enzymes is not increased although their total quantity is considerably increased (p. 486). Another hormone, pancreozymin, secreted by the duodenal mucosa, has an effect similar to that produced by vagal stimulation, but not mediated by the vagus.

Vagus stimulation results in a pancreatic secretion of small volume with an extremely high enzyme content. The vagus effect may be produced by administration of insulin (hypoglycemia), mecholyl chloride, pilocarpine or prostigmine.

EXAMINATION OF PANCREATIC JUICE · SECRETIN TEST

Quantitative analysis of the pancreatic juice should afford a rational and accurate method of studying the external secretory function of the pancreas. Use of a double-lumen gastroduodenal tube, properly placed under fluoroscopic guidance, has largely overcome the difficulty of obtaining pancreatic juice uncontaminated by variable amounts of gastric juice. A more or less pure pancreatic juice is obtained by stimulation with a purified preparation of secretin.

The long end of the tube is passed to the third portion of the duodenum, in which position the shorter end (ten inches shorter) is in the stomach. Continuous gentle suction is then applied, the negative pressure not exceeding 50 mm. Hg, and the gastric juice and duodenal contents are collected simultaneously. Usually after about twenty to twenty-five minutes, the duodenal juice becomes clear and is no longer contaminated with gastric juice. The duodenal contents consist principally of pancreatic juice and usually some bile. When admixture with gastric juice has ceased a twenty-minute basal sample is obtained, and secretin is injected intravenously, in a dosage of 1 unit of a commercial preparation per kilogram of body weight. Collection is continued for 1 hour, separated, if desired, into ten- or twenty-minute samples.

Immediately after injection of secretin, the volume of material aspirated from the duodenum increases strikingly, usually with a change in color. Prior to injection this is usually light yellow-brown unless the gallbladder is emptying. Promptly after stimulation of pancreatic secretion, either the color becomes paler or all trace of bile pigment disappears. In most instances the duodenal sphincter appears to remain closed and the liver bile accumulates in the gallbladder during the experimental period. The occurrence of a deeply bile-stained secretion throughout the test period suggests that the gallbladder is absent, diseased or nonfunctioning. Regurgitation of duodenal contents into the stomach occurs occasionally, especially in cases of achylia gastrica, when the pyloric mechanism is incompetent. This is evidenced by a sudden rise in the volume of a gastric fraction, by bile discoloration and by reduction in acidity of the fraction. By measuring the volume and the concentrations of bicarbonate and bilirubin, and comparing the latter with their concentrations in the corresponding duodenal fraction, the quantity of regurgitated material may be estimated with reasonable accuracy. The following quantitative determinations are made: (1) volume; (2) bicarbonate; (3) amylase; (4) trypsin; (5) lipase. Values are expressed as quantities excreted in the one-hour test period. Amylase is measured by the activity of digestion of starch, trypsin by digestion of protein (casein) and lipase by digestion of a fat substrate (e.g., olive oil). These are commonly expressed in terms of units of digestive activity.

Volume. In normal subjects, 135 to 250 cc. of pancreatic juice are obtained in the sixty-minute period, or 2.1 to 4.5 cc. per kilogram of body weight. In the majority of instances maximum secretion occurs during the first ten to twenty minutes, with a subsequent rapid fall, approximating the control rate of secretion in sixty to one hundred twenty minutes. The maximum rate of secretion varies from about 2 to 8 cc. per minute.

Bicarbonate. The normal total bicarbonate output in the one-hour period is 90 to 130 mEq., the maximum concentration occurring usually in twenty to forty minutes and falling more slowly than does the volume.

Amylase (Diastase). About 300 to 1200 units of amylase are excreted in the one-hour period (5.5–11 units per kilogram of body weight). The curve of excretion reaches a peak during the first twenty minutes, falls sharply after twenty minutes and then gradually during the next one to two hours. The concentration of the enzyme is lower than in the resting juice except during the first ten minutes, during which the pancreas is probably emptying its store of preformed amylase.

Subsequently, the stimulated gland forms a rather constant amount of enzyme, independent of the volume of secretion.

Trypsin. About 20 to 40 units are excreted normally in one hour (0.35–0.7 units per kilogram of body weight). What has been said of amylase applies to trypsin.

Lipase. The normal one-hour excretion is 7000 to 14,000 units (135–225 units per kilogram of body weight).

This procedure has certain practical limitations. The double-lumen tube is comparatively large and produces a variable degree of discomfort. The tips must be placed properly under fluoroscopic guidance, and even then varying amounts of gastric juice may enter the duodenum and contaminate the samples of pancreatic juice. There is evidence that pancreatic secretion may be stimulated mechanically by the presence of the tube in the duodenum, contributing to the rather wide variation in values obtained in normal subjects. Moreover, there is difficulty in obtaining uniformly active preparations of secretin suitable for intravenous injection.

Abnormal Findings with the Secretin Test

The general statement may be made that in case of obstruction of the pancreatic duct or extensive destruction of pancreatic tissue (e.g., acute necrosis, carcinoma or fibrosis) there is usually a decrease in values for volume, bicarbonate and enzymes, under the conditions of the test, the decrease being proportional to the degree of duct obstruction or extent of tissue destruction. However, whereas this may be true of advanced lesions, such findings are not commonly observed in cases of mild pancreatic dysfunction. The earliest functional abnormality appears to be reflected usually in decreased excretion of amylase and lipase and later by a decrease in trypsin, the volume and particularly the bicarbonate being less easily disturbed. Nondissociated disturbance of function, as evidenced by a decrease in all factors, may perhaps be interpreted as indicating obstruction of the pancreatic duct or a significant diminution in the mass of functioning pancreatic tissue. Dissociated disturbance, first consisting usually in a decrease in amylase or lipase, the other factors being relatively normal, represents a milder form of functional disturbance, and is seen characteristically in mild forms of acute and chronic pancreatitis.

Abnormal findings have been obtained with this procedure in patients with acute and chronic pancreatitis, pancreatic cysts, hemochromatosis, cystic fibrosis, calcification, and carcinoma of the pancreas and pancreatic edema. Abnormal results, indicating clinically unsuspected complicating pancreatic disturbance, have been obtained also in patients with diabetes mellitus, cholelithiasis, cirrhosis of the liver, acute hepatic necrosis and late syphilis. The procedure is of value in distinguishing steatorrhea of pancreatic origin from the idiopathic form (sprue, celiac disease), in which the secretin test yields normal results.

The response to insulin, pancreozymin, and mecholyl chloride has been utilized in the same manner as the secretin test. These agents normally produce a marked increase in the output (concentration) of pancreatic enzymes, the bicarbonate being unaffected. They have also been given in conjunction with secretin, the resulting output of enzymes being much greater than with secretin alone. However, the findings in abnormal states appear to parallel those obtained with secretin alone, and the use of these agents adds little information of diagnostic value.

The chief disadvantages of the secretin test as a routine diagnostic procedure are the practical limitations referred to previously. Findings within the normal range are commonly obtained in mild pancreatitis. Although advanced disease is usually reflected in moderate or severe impairment of acinar cell function, as indicated by this procedure, it does not provide information of value in differential diagnosis, similar findings being obtained in pancreatic fibrosis, cysts, calcification, edema, inflammation, carcinoma, duct obstruction, etc.

EXAMINATION OF THE FECES

The determination of the presence or absence of pancreatic enzymes in the feces is of no practical significance. Perhaps the most valuable methods for the study of pancreatic function in the past have had for their basis the examination of the feces for evidence of inefficient fat and protein digestion. Fat is normally present in the feces in three forms, soap fat (combined fatty acid), free fatty acids and neutral fat, the relative proportion of each being dependent somewhat upon the efficiency of fat digestion and absorption. The normal values for each of these forms have been given elsewhere (p. 96).

Since pancreatic lipase splits neutral fat into fatty acids, the point of particular importance from the standpoint of the diagnosis of pancreatic functional efficiency should be the amount of unsplit fat present in the feces and the proportion this constitutes of the total fecal fat. From a consideration of the results obtained in a large series of cases Fowweather has reached the following conclusions:

(1) Any specimen in which the total fat amounts to more than 25 per cent of the total dry matter is probably abnormal. The presence of excessive amounts of fat in the feces is termed "steatorrhea."

(2) Any specimen in which the neutral fat exceeds 11 per cent of the total dry matter or 55 per cent of the total fat should be suspected of showing evidence of deficient fat splitting.

(3) Any specimen in which the total split fat (i.e., sum of soap fat and free fatty acids) exceeds 16 per cent of the total dry matter or 75 per cent of the total fat should be suspected of showing evidence of deficient fat absorption.

The following is an example of the results obtained by the analysis of fecal fat in chronic pancreatitis:

	Per cent of total dry matter
Total fat	42.1 (normal 17.5)
Soap fat (combined fatty acids)	11.0 (normal 4.6)
Free fatty acids	5.8 (normal 5.6)
Neutral fat	24.4 (normal 7.3)

The characteristic finding in this connection in pancreatogenous steatorrhea is a predominating increase in neutral fat, indicating deficient fat splitting in the intestine. Characteristically, in severe cases, the feces are pale, bulky and foul-smelling, and, when allowed to stand, droplets or solid masses of neutral fat may be visible. This does not occur in celiac disease, sprue or idiopathic steatorrhea, in which the major increase in fecal fat occurs typically in the fatty acid fraction because of inadequate absorption. However, the findings are so similar in many cases of pancreatitis, cystic fibrosis, celiac disease, sprue, and idiopathic steatorrhea as to make the differentiation between pancreatitis and the other conditions impossible on this basis. Excessive loss of neutral fat (more than 35–40%) points to pancreatic insufficiency. The secretin test (p. 485) is of greater value in the differential diagnosis of these conditions. Excessive amounts of undigested material, including fat, may be found in the feces of subjects with gastrocolic fistula.

In the presence of jaundice associated with pancreatic disease the values for all of the fatty constituents of the feces are considerably increased, the total fat in some cases constituting as much as 75 per cent of the total dry matter. In such cases there is evidence of disturbance of both fat digestion and fat absorption. Disturbances of intestinal motility, particularly those characterized by the rapid transit of food through the small intestine, are associated with alterations in the fat content of the stool. However, in such cases the fault lies primarily and principally in deficient fat absorption, and the proportion of neutral fat is usually within normal limits.

As stated elsewhere, doubt has been cast upon this interpretation of variations in the relative proportions of the fatty constituents of feces due to the demonstration that fats are excreted in relatively large amounts by the intestinal mucosa. It appears probable, for example, that the enormous increase in fecal fat in obstructive jaundice may be due largely to an increased excretion of fat rather than to diminished absorption, as was formerly believed to be the case. Moreover, as the excreted endogenous fat is in the form of neutral fat and fatty acids, it is obviously impossible to interpret alterations in the relative proportions of these substances in the feces in terms of altered digestion and absorption of ingested fat.

The plasma vitamin A concentration (normal 10–60 μg./100 cc.) is usually low in children with cystic fibrosis of the pancreas. Very little or no increase occurs after oral administration of 5000 I.U. (approximately 1000 μg.) of vitamin A in oil, in contrast to a normal rise to levels of 200–600 μg./100 cc. A normal increase follows ingestion of the vitamin emulsified in a water-propylene glycol mixture. These findings reflect the difficulty in fat absorption in this condition. Similar findings are obtained in celiac disease, sprue, and obstructive jaundice.

Protein in Feces. In the normal individual not more than 5 to 10 per cent of the nitrogen of the food is lost in the feces. The term "azotorrhea" is used to indicate the excretion of excessive quantities of nitrogenous compounds in the feces, a condition dependent upon either imperfect digestion of proteins or imperfect absorption of protein derivatives. In the absence of abnormalities of absorption or intestinal motility, azotorrhea may be suggestive of pancreatic functional inefficiency. The presence in the feces of 25 per cent or more of the nitrogen of the food is frequently observed in pancreatic disease, whereas values above 17 per cent are rarely observed in sprue or idiopathic steatorrhea. In patients with steatorrhea maintained on a diet low in fat, a daily excretion of more than 3 gm. of nitrogen suggests pancreatic dysfunction. Azotorrhea may also occur in subjects with gastrocolic fistula.

The stools of normal individuals may contain a few meat fibers with rounded ends and no transverse striations. Pancreatic enzymatic activity appears to be essential for digestion of meat fibers to proceed to the point at which the transverse striations disappear. Creatorrhea is a term applied to the presence of excessive quantities of undigested meat fibers in the feces, the fibers having sharp angular ends and transverse striations. In the presence of normal intestinal motility, creatorrhea is suggestive of impaired pancreatic function. In order to obviate, so far as is possible, misinterpretations due to too rapid or too slow passage of material through the intestines, all purgatives must be withheld and carmine and charcoal marked meals administered, only feces passed within from eighteen to thirty hours after ingestion of the corresponding food being accepted for examination. Abnormal findings on microscopic examination because of their qualitative nature, are not in themselves diagnostic, but should serve as an indication for more exact chemical studies of the feces and for other tests of pancreatic function.

Serum Amylase (Diastase). Blood normally contains a starch-splitting enzyme (amylase, diastase), which is also present in urine, lymph, feces and milk. Although apparently identical with salivary and pancreatic amylase, its origin is not known, since it is maintained at an approximately normal level after extirpation of the pancreas and salivary glands. However, certain acute pathologic changes in these glands may result in temporary diffusion of amylase into the blood. This phenomenon has proved of value in the diagnosis of acute pancreatitis. The amylase content (amylolytic or diastatic activity) of the blood plasma or serum is usually expressed in units which represent the milligrams of glucose formed by digestion of the starch substrate. The diastatic activity of normal serum or plasma (Somogyi method) is 60 to 180 units per 100 cc., 80 per cent of normal subjects falling between 80 and 150 units. Values above 200 units and below 60 units are abnormal.

The chief clinical value of serum amylase determinations lies in the diagnosis of acute pancreatitis, whether the lesion is of the edematous, hemorrhagic, suppurative or necrotic type. In this condition, the serum amylase practically invariably increases almost simultaneously with the onset of symptoms, usually rising above 500 units and occasionally to over 3000 units, the peak being reached usually in 12 to 24 hours, but occasionally as late as 48 hours. After reaching the peak, there is usually a precipitous but occasionally a gradual fall to a normal or subnormal level within 2 to 6 days after the onset. The absence of an increase in serum amylase within the first 6 to 24 hours after the onset of acute symptoms almost certainly excludes the possibility of acute pancreatitis as the cause of the symptoms, except in rare instances of extremely rapid and extensive destruction of acinar cells. Because of the rapidity with which normal values are restored, negative findings after 48 to 72 hours do not exclude this possibility. With the possible exception of serum lipase determinations, this procedure constitutes the most valuable method available for the diagnosis of acute pancreatitis. The increase is probably due mainly to inflow of preformed amylase from the damaged pancreas directly into the circulation via the portal vein and to a smaller extent via the lymphatics and thoracic duct.

An increase in serum amylase has been reported in cases of peptic ulcer perforating into the pancreas, the values being considerably lower than in acute pancreatitis. Moderate increase has been observed occasionally in certain diseases of the salivary glands, including mumps, suppurative parotitis and calculous obstruction of the salivary duct. Moderate elevation (200–1000 units) may occur also as a result of renal functional impairment, due to deficient excretion of amylase. Subnormal values have been reported in subjects with severe hepatocellular damage (p. 549).

Normal findings are the rule in chronic pancreatic lesions, e.g., carcinoma, atrophy, cysts, fibrosis, calcification. However, values comparable to those in acute pancreatitis are observed rather consistently during acute exacerbations in chronic recurrent (relapsing) pancreatitis and, occasionally, in carcinoma of the head of the pancreas (duct obstruction).

Serum Amylase After Stimulation. Administration of secretin alone does not result in an increase in serum amylase in normal subjects. This does occur, however, if morphine sulfate is given simultaneously (spasm of sphincter of Oddi). An increase occurs in normal individuals also after subcutaneous injection of mecholyl chloride (10 mg.) or of urecholine (5 mg.). This is probably due to simultaneous stimulation of enzyme secretion by the pancreas and constriction of the ampullary spincter by these agents. This procedure may be combined with intravenous secretin administration.

Blood samples for amylase determination are obtained before, and 15, 30, 60, and 90 minutes (or merely 30 and 60 minutes) after injection. Average increase of as much as 200 per cent above the basal level has been observed in normal subjects, the peak occurring usually at 30 to 60 minutes. The largest increments are observed following simultaneous injection of secretin and mecholyl chloride. Abnormally large increases have been reported in the presence of normal basal values in certain cases of pancreatic duct obstruction, subsiding acute pancreatitis, and chronic pancreatitis. Subnormal increments have been reported in pancreatic carcinoma, fibrosis, cystic disease, and in chronic pancreatitis. However, such findings are by no means consistent, and these procedures have not been employed sufficiently extensively to establish the limits of normal response. When methods have been adequately standardized, they may prove to be of clinical value in the diagnosis of chronic pancreatic disorders.

SERUM LIPASE

The degree of lipolytic activity of the serum may be determined by the amount of olive oil hydrolyzed by a given quantity of serum in a given time. According to the titration method advocated by Comfort, the values for lipase are expressed in terms of the amount of N/20 NaOH per 1 cc. of serum. The upper limit of normal has been found to be about 1.5 cc. of N/20 NaOH.

Increase in serum lipase is believed to be indicative of pancreatic disease, the enzyme entering the blood stream in the same manner as pancreatic amylase. The findings with regard to lipase differ in certain important respects from serum amylase in pancreatic disease. As in the case of the latter, in acute pancreatitis the serum lipase increases promptly at the time of onset of symptoms, values as high as 10.2 cc. having been reported. The subsequent fall is much more gradual than in the case of amylase, elevated values persisting in some cases for ten to fourteen days or longer. Because of this fact, accurate diagnosis is often possible by means of lipase determinations after the serum amylase has fallen to an essentially normal level. This procedure is preferred by some for this reason. In contrast to the usual normal levels of serum amylase in chronic disease of the pancreas, increase in serum lipase has been reported in about 40 to 50 per cent of cases of carcinoma of the pancreas, about 60 per cent of cases of carcinoma of the ampulla of Vater, 10 to 15 per cent of cases of chronic biliary tract disease (common duct stone or stricture, carcinoma of the bile ducts, cholangitis), and occasionally in duodenal ulcer, hepatitis and cirrhosis of the liver and chronic pancreatitis or pancreatic duct obstruction. In conditions not primarily involving the pancreas, increase in serum lipase is believed to indicate secondary pancreatic involvement.

AMYLASE IN URINE

In the absence of significant impairment of renal function, variations in amylase in the urine tend to parallel those in the blood. Usually, in acute pancreatitis, the urine values rise shortly after those in the blood, the increase persisting for one to three days, and then fall sharply to normal. Values as high as 8000 units (normal, 5–50 units/cc.) have been reported in acute pancreatitis, 300 units or more being of diagnostic significance. Elevated values have been obtained in chronic pancreatitis (during acute exacerbations), stone in the common bile duct, duodenal ulcer perforating into the pancreas, obstruction of the pancreatic duct and occasionally in carcinoma of the pancreas.

The following facts should be kept in mind in connection with amylase findings in blood and urine:

(*a*) As in the case of serum amylase, much of the difference of opinion regarding the value of urinary diastase determinations has been due to failure to appreciate the transitory nature of the increase in acute pancreatitis.

(*b*) Excluding disease of the salivary glands and, in the case of serum amylase, renal insufficiency, an increase is indicative of pancreatic disease, usually acute, but this may be primary, or secondary to or associated with such conditions as stone in the common duct, tumor of the common duct, stomach or duodenum, hepatic disease or duodenal ulcer.

(*c*) These procedures give no information regarding the nature or severity of the pancreatic lesion.

(*d*) Normal values do not invariably rule out acute pancreatitis, since they may be obtained when rapid extensive destruction of acinar cells has occurred.

MISCELLANEOUS FINDINGS IN PANCREATIC DISEASE

The occurrence of hyperbilirubinemia in carcinoma of the head of the pancreas is referred to elsewhere (p. 525). It occurs also occasionally in patients with acute and chronic pancreatitis, probably reflecting associated biliary tract or hepatic disease.

Glucose tolerance is decreased in many cases of pancreatic carcinoma, particularly when the body and tail are involved, and also in chronic (about 30 per cent) and acute pancreatitis. Fasting hyperglycemia has been observed in 15 to 100 per cent of different series of cases of acute pancreatitis, with glycosuria in 5 to 25 per cent. The incidence is lower in chronic pancreatitis (hyperglycemia in 15 to 30 per cent).

Hypocalcemia has been reported in acute pancreatitis (up to 70 per cent), developing usually 24 to 72 hours after the acute attack (p. 239). The decrease is of slight degree (8–8.5 mg./100 cc.) in the majority of cases, but values below 7 mg./100 cc., accompanied by tetany, are observed occasionally. This has been attributed to sudden withdrawal of large amounts of calcium from the extracellular fluids by fixation as calcium soaps (i.e., by fatty acids) in areas of fat necrosis (p. 240).

A type of chronic relapsing pancreatitis has been described which is associated with an increase in plasma lipids, mainly neutral fat, but also cholesterol and phospholipids. Other manifestations of hyperlipemia may be present, viz., skin xanthomatosis and lipemia retinalis, and the recurring acute attacks resemble those in idiopathic familial hyperlipemia (p. 119). The pancreatic involvement, as well as other manifestations, have been attributed to fat embolization due to change in the physical state of the plasma lipids. The hyperlipemia often persists during symptom-free periods.

Disturbance of the sweating mechanism has been observed in young children with fibrocystic disease of the pancreas, characterized by unusually profuse perspiration in hot weather. There is a marked increase in the Na and Cl content of the sweat, which does not exhibit the normal decrease following administration of desoxycorticosterone. This abnormality predisposes to the development of heat prostration in such cases.

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CHAPTER XXI

Hepatic Function

The determination of the functional efficiency of the liver by chemical methods, or in fact by any laboratory procedure, offers unusual difficulties for several reasons. One of these is the fact that whereas the multiplicity of duties performed by the liver may in certain cases be equally involved in the presence of hepatic disease, it is probable that in some cases one or more of these functions may be disturbed relatively more than the others. Then, too, in several aspects of its functional activity the liver is so intimately associated with other organs that, in investigating these functions, it is difficult or impossible, as stated by Mann, to delineate definitely the hepatic factor. Moreover, the liver possesses an enormous functional reserve and remarkable regenerative powers. It has been demonstrated that within a few weeks after the removal of an entire lobe the liver may be as large as before the operation. Furthermore, by means of consecutive operations and by the institution of measures designed to inhibit this regenerative tendency, the total hepatic tissue may be permanently reduced to as low as 15 per cent of the normal amount without remarkable impairment of hepatic functional efficiency if a proper dietary regime is maintained. Under such circumstances, however, the liver is not able to withstand untoward conditions as well as in its normal state. This experimentally demonstrated fact may explain the frequent failure to demonstrate functional impairment even in the presence of advanced anatomic changes which may be either primary in the liver or secondary to lesions in the extrahepatic biliary passages. This is particularly true of chronic conditions such as cirrhosis, syphilis, malignancy, and the like, which, because of their relatively slowly progressive nature, are associated with concomitant compensatory regeneration of hepatic tissue with little or no impairment of hepatic functional efficiency. In acute widespread hepatic disease, such as occurs in acute yellow atrophy of the liver, arsphenamine hepatitis, and phosphorus and chloroform poisoning, the investigation of liver function yields more consistently positive findings.

In spite of the frequently negative findings, valuable information may at times be obtained in chronic diseases of the liver and biliary passages by the utilization of certain well established methods of investigating liver function. The routine employment of such procedures is of particular importance as a part of the preoperative study of patients with biliary tract disease, for by the institution of measures designed to increase hepatic functional efficiency, much can be done to diminish postoperative morbidity and mortality in these conditions. The fact is too frequently overlooked that functional tests are designed to indicate the state of functional activity of an organ and not necessarily the presence or extent of morphologic changes in that organ. Obviously, it is possible that an organ such as the liver may be the seat of extensive and even grossly evident chronic disease, and still may be capable of carrying on its functions in an essentially normal manner; conversely, it is also conceivable that the functional activity of an organ may be impaired in the absence of demonstrable morphologic changes. If these facts are kept in mind, results obtained by the application of tests of liver function will have considerable clinical significance. On the other hand, any attempt to interpret functional findings in terms of disease diagnosis or of the extent of hepatic parenchymal change may lead to serious error.

CARBOHYDRATE METABOLISM

The important part played by the liver in normal carbohydrate metabolism has been considered elsewhere in detail (p. 8). Glucose, preformed or resulting from intestinal digestion of carbohydrates, reaches the liver through the portal circulation. The portion which is not utilized immediately for the maintenance of body activities is stored chiefly in the liver, as glycogen which, when the necessity arises, is converted to glucose which passes in the general circulation to the tissues. Liver glycogen is also contributed to from two other sources: (1) glucose resulting from gluconeogenesis from protein, a process which takes place in the liver and is influenced by hormones of the adrenal cortex; (2) lactic acid, resulting from breakdown of glycogen in the muscles, passing in the blood to the liver, where it undergoes retransformation into glycogen under normal conditions of hepatic function and insulin action. The glycogen in the liver constitutes the reserve for the maintenance of the normal glucose concentration of the blood. This function of hepatic glycogen is not shared by glycogen deposits elsewhere in the body, for, following experimental extirpation of the liver, hypoglycemia occurs which rapidly progresses to a fatal termination although relatively large quantities of glycogen may be present at the time in the muscles.

The influence of the liver in carbohydrate metabolism depends upon the functional activity of the hepatic cells and is not affected by obstruction to the flow of bile per se. Disturbance of hepatic cell function, as indicated below, may result in (1) a tendency toward fasting hypoglycemia, (2) diminished glucose, fructose and galactose tolerance, (3) diminished blood sugar response to epinephrine, (4) glycosuria and (5) increased blood lactic acid concentration and diminished lactic acid tolerance. The presence of such abnormalities in a patient with jaundice suggests the existence of impaired liver cell function, presumably due to hepatic cell damage, while their absence suggests that hepatic function is normal (uncomplicated obstructive or hemolytic jaundice). These procedures have therefore been employed for the purpose of distinguishing between hepatocellular and obstructive types of jaundice. However, clinical obstructive jaundice, particularly if due to cholelithiasis, is usually accompanied, sooner or later, by a variable degree of hepatocellular damage. Moreover, hyperglycemia, diminished glucose tolerance and glycosuria may be contributed to by associated pancreatic disease, and the possibility of other, unrelated disturbances of carbohydrate metabolism must be taken into consideration. Because of these facts, the value of studies of carbohydrate metabolism in differentiating obstructive from hepatocellular jaundice is distinctly limited, and the results must be interpreted in the light of other findings. This is less true of studies of galactose and lactic acid metabolism than of glucose metabolism.

Fasting Blood Sugar Level. Because of the extensive functional reserve and enormous regenerative capacity of the liver the fasting blood sugar is usually found to be within normal limits in hepatic disease, even though the hepatic glycogen content may be markedly depleted. This is particularly true of chronic disorders such as portal cirrhosis, biliary cirrhosis, syphilis, carcinoma, etc. Values of 50 to 60 mg. per 100 cc. may at times be observed in the terminal stages of cirrhotic processes, particularly in obstructive biliary cirrhosis but occasionally also in portal cirrhosis. Serious and occasionally fatal hypoglycemia may occur after operation for biliary tract disease under general anesthesia, which may exhaust the glycogen reserve already diminished by associated but not readily detectable hepatic disease (hepatitis). Hypoglycemia is more commonly observed in acute, extensive forms of hepatic disease. Values ranging from 25 to 60 mg. per 100 cc. have been reported in cases of phosphorus, cinchophen, chloroform and carbon tetrachloride poisoning and in arsphenamine hepatitis. Acute yellow atrophy of the liver (acute diffuse necrosis) and yellow fever are at times associated with extremely low values (15–50 mg.) which may be an important factor in the fatal termination of such cases. It must be realized, however, that the absence of hypoglycemia, even in these acute disorders, is by no means indicative of the absence of hepatic functional impairment. When one realizes the fact that 80 per cent of the total substance of the liver may be removed without producing hypoglycemia, one can appreciate readily why this occurs in extensive hepatic disease only as a terminal event.

The phenomenon of spontaneous hypoglycemia, occurring at times paroxysmally, has also been observed in patients with extremely fatty liver, extensive hepatic cirrhosis and widespread involvement of the liver with carcinoma. The occasional occurrence of temporary periods of hypoglycemia in apparently normal individuals subjected to extremely strenuous exercise, such as marathon running, is due perhaps to temporary exhaustion of the hepatic glycogen reserves. As a result, the balance between the utilization of glucose in the muscles and its liberation into the blood stream from the liver is disturbed, and the blood sugar falls unless glucose is supplied from external sources. Whereas the superimposition of hepatic disease usually further diminishes the already lowered glucose tolerance of diabetics and increases their resistance to insulin, the opposite is observed occasionally. Patients with diabetes have been observed in whom the development of extensive parenchymal damage, as in hemochromatosis, was followed by paroxysmal hypoglycemia.

Cases of biliary tract and hepatic disease are encountered occasionally in which the fasting blood sugar concentration is above normal, returning to normal after improvement in the biliary tract condition. This is explained by some on the basis of a disturbance of the mechanism which regulates the rate of breakdown of glycogen in the liver in accordance with the utilization of glucose in the tissues and the supply of glucose to the liver (p. 22). However, great care must be exercised in such cases in eliminating the possibility of the existence of a state of latent diabetes aggravated by the presence of hepatic disease.

Glucose Tolerance. When the glycogen storing function of the liver becomes seriously impaired, the blood sugar curve following the ingestion of glucose exhibits an abnormally high rise and relatively delayed fall. If the blood sugar concentration rises above the "renal threshold level" glucose will appear in the urine. In the presence of advanced hepatic disease, particularly in acute conditions, decreased glucose tolerance may frequently be demonstrated. The blood sugar curve usually differs from that characteristically observed in diabetes in that, in contrast to the latter condition, there is a tendency toward fasting hypoglycemia with a relatively rapid rise and fall of the blood sugar level following the ingestion of glucose. If sugar determinations are made upon both arterial and venous blood following the ingestion of glucose, it may be found that although there is an excessively high rise in both arterial and venous blood sugar, the arterial-venous difference remains within normal limits or may in some cases be increased. This may aid in differentiating between the diminished sugar tolerance of deficient hepatic glycogenesis and that of diabetes, in which condition the arterial-venous difference is characteristically decreased. The curve obtained in typical cases of hepatic insufficiency is characterized by:

(*a*) Normal, elevated or subnormal fasting blood sugar level.

(*b*) Abnormally high rise in venous blood sugar concentration (above 160 mg.).

(*c*) The maximum concentration is attained at the end of $\frac{3}{4}$ to $1\frac{1}{2}$ hours, in most instances within 1 hour.

(*d*) The blood sugar usually falls rather rapidly and returns to the fasting level within two to three hours. Only in cases of extreme grades of hepatic insufficiency is hyperglycemia of long duration.

(*e*) The arterial-venous difference is usually normal, or in some instances increased.

(*f*) Because of the absence of disturbance of utilization of glucose in the tissues, the respiratory quotient and serum phosphate change in an essentially normal manner following the administration of glucose; i.e., the respiratory quotient rises and the serum phosphate falls during the period of increased glucose supply and utilization.

It has been suggested that failure of the glycogen-storing mechanism of the liver may not be the only factor responsible for the production of diminished glucose tolerance in hepatic disease. Some believe that under normal conditions a decreased output of sugar from the liver (decreased hepatic glycogenolysis) is an important although not necessarily the only factor in determining the characteristic fall in the blood sugar curve after the administration of glucose. In the presence of hepatic functional impairment there may be some interference with the mechanism whereby the liver normally diminishes its supply of glucose to the blood in response to an influx of exogenous sugar. According to this hypothesis, the deleterious influence of diminished hepatic glycogenesis in contributing to the production of diminished glucose tolerance is supplemented by increased or abnormally prolonged hepatic glycogenolysis, the result being an abnormally high and prolonged elevation of blood sugar following the ingestion of glucose.

Although such findings are commonly obtained in patients with hepatic disease, the determination of glucose tolerance has proven to be of little or no practical value in the study of hepatic function. So many factors are involved in the intermediary metabolism of glucose that the interpretation of minor changes in the blood sugar tolerance curve is extremely difficult. Furthermore, because of the activity of extrahepatic factors in the storage and utilization of glucose, essentially normal findings may be obtained occasionally in the presence of advanced hepatic disease.

A peculiar type of disturbance of glucose tolerance has been described in the condition known as glycogen disease (von Gierke's disease) (p. 57). This is apparently a disturbance of glycogen metabolism which appears in early infancy and is characterized by an abnormal deposit of glycogen in the liver, kidneys, heart and other organs, which become so engorged with glycogen that they assume an enormous size. The peculiarity of this store of glycogen is that it becomes fixed in some way and cannot be mobilized to any extent under ordinary conditions. Because of the extraordinary avidity with which the liver and other tissues remove glucose from the blood and store it as glycogen, together with the abnormal stability of the latter, resulting in diminished glycogenolysis, the fasting blood sugar concentration may be subnormal, values as low as 20 mg. per 100 cc. having been reported. Due to the operation of the same factors, the alimentary blood sugar response exhibits a more gradual and less marked increase than normal and, frequently, a more delayed return to the fasting level. The latter phenomenon is difficult to explain.

Epinephrine Hyperglycemia (see pp. 35, 54). The increase in blood sugar which follows the administration of epinephrine in normal individuals apparently depends upon the presence of adequate amounts of glycogen in the liver. Following the administration of 10 minims of a 1 : 1000 solution of epinephrine hydrochloride by intramuscular injection, the blood sugar concentration normally increases 35 to 45 mg. per 100 cc. in three-fourths to one hour and returns to the resting level in one and three-fourths to two hours. Several observers have noted that in the presence of hepatic disease the degree of rise in the blood sugar concentration is not as great as that observed in normal individuals. In some cases of advanced functional impairment associated with acute hepatic disease practically no rise is obtained. These phenomena are presumably due to the diminution in or depletion of the glycogen reserve in the liver. This test usually yields but little information of value in the early diagnosis of liver disease. Variable results are obtained in chronic liver disease, particularly in portal cirrhosis, although good results have been reported in cases of obstructive biliary cirrhosis. Decrease in or absence of the normal response to epinephrine may frequently be observed in patients with acute diffuse hepatic lesions, such as toxic hepatitis, phosphorus and arsenic poisoning, acute and subacute yellow atrophy of the liver, etc. A diminished blood sugar response to epinephrine occurs also in patients with glycogen disease (von Gierke's disease) (p. 57).

Fructose Tolerance. On the basis of the observation that following hepatectomy in frogs the metabolism of fructose may be disturbed whereas that of glucose is relatively unaffected, Strauss introduced the fructose tolerance test as a method of estimating liver function. The procedure as originally described consists in the administration of 100 gm. of fructose by mouth and the subsequent examination of the urine for fructose over a period of six hours following the ingestion of the sugar. Strauss believed that about 80 per cent of patients with liver disease exhibit a diminished tolerance for fructose as evidenced by the appearance of this substance in the urine under these conditions. About 10 per cent of normal individuals react positively to this test. At the present time this procedure is believed to be of little practical value. Negative results are obtained in many patients with hepatic disease. Moreover, the renal threshold for fructose is apparently much lower than that for glucose, the exact level being unknown and varying within wide limits in normal individuals, thus rendering the interpretation of positive results extremely difficult. This procedure has been largely supplanted by methods which include the determination of the blood sugar concentration at intervals following the administration of fructose.

FRUCTOSE TOLERANCE TEST. A sample of blood is withdrawn in the fasting state. Forty-five gm. of fructose are given by mouth in 200 cc. of water or lemonade. Sugar determinations are made upon samples of blood withdrawn at one-half hour intervals for two hours. In normal individuals the blood sugar rises 15 mg. per 100 cc. or less and rarely exceeds 130 mg. per 100 cc. Hepatic functional impairment is evidenced by the following phenomena:

(*a*) A rise in blood sugar of more than 35 mg. per 100 cc. At least one blood sugar reading above 135 mg.

(*b*) Failure of the blood sugar curve to return to the resting level in two hours.

Although results obtained by this test appear to be more satisfactory than those obtained by the glucose tolerance test, it has proved to be of little practical value in the early diagnosis or quantitative estimation of hepatic functional impairment. Positive results are obtained more frequently in the presence of acute lesions of the hepatic parenchyma than in diseases of the extrahepatic biliary passages. In most instances this test yields no information which cannot be obtained more readily and more satisfactorily by other methods. More satisfactory results have been reported when the procedure is modified so that the blood fructose alone is determined rather than fructose plus glucose. Normally the blood fructose concentration, 0 to 8 mg. per 100 cc. in the fasting state, increases not more than 15 mg. per 100 cc., usually within the first hour after ingestion of 50 gm. of fructose, and falls to 0 to 10 mg. within two hours. Increases of 16 to 30 mg. with a delayed fall to the resting level have been observed in patients with hepatocellular damage and occasionally also in arteriosclerosis.

Galactose Tolerance. Galactose, which enters the sphere of glucose-glycogen metabolism practically exclusively in the liver, has been used to test the efficiency of the glycogen-storing mechanism of the liver. The most commonly employed tests of galactose tolerance are concerned merely with the elimination of this substance in the urine.

In the cases of females, 40 gm., and in males, 30 gm. of galactose are administered in 250 to 500 cc. of cold water in the fasting state. Urine is collected hourly for five hours following the ingestion of galactose. Each test sample is examined for reducing substances by the Benedict qualitative reagent; if positive, the quantitative Benedict test is performed. Melituria of less than 3 gm. is of no practical significance. The elimination of more than 4 to 5 gm. in the five-hour period is assumed to indicate hepatic functional impairment. Many observers believe that the galactose test gives positive results in infectious, toxic, and other types of hepatitis and hepatic necrosis, being negative in simple obstructive jaundice. It has been found by some that circumscribed liver affections do not diminish the tolerance for galactose unless infection coexists. Negative results are usually obtained in cirrhosis and passive congestion of the liver. In view of these observations, the galactose tolerance test has been suggested as a means of differentiating hepatic parenchymal disorders such as infectious, toxic, and other forms of hepatitis (hepatocellular jaundice) from obstructive jaundice. It has also been advocated as a means of detecting hepatic parenchymal damage occurring as a complication of obstructive jaundice.

Although several investigators regard the galactose tolerance test as of value in differentiating between extrahepatic obstructive and hepatocellular types of jaundice, our own experience and that of the majority of observers may be summarized as follows: (1) Although a positive galactose test is almost the rule in cases of acute hepatocellular jaundice of average severity, a negative response may occur in mild or convalescent cases or in cases of long standing, and does not necessarily militate against the diagnosis. (2) A positive response may be obtained in 30 to 45 per cent of patients with obstructive jaundice of various types. (3) The galactose tolerance test does not uniformly distinguish between obstructive and hepatocellular types of jaundice, although it may furnish corroborative data valuable in doubtful cases. (4) In both varieties of jaundice a strongly positive test (excretion of 6 gm. or more) should be regarded seriously and, if excretion is continuously high, is probably indicative of serious hepatic parenchymal injury. The border-line group of positive tests, with excretion of only slightly more than 3 gm. of reducing substance, should not be regarded as conclusive evidence against the presence of mechanical biliary obstruction. The occurrence of positive results by this test in patients with extrahepatic biliary obstruction is not difficult to understand when one recalls the extensive morphologic changes that may and frequently do occur in the liver cells under such circumstances.

Studies of the blood galactose curve after administration of galactose yield results that may be more useful in this connection. In the absence of hyperthyroidism (p. 56), blood galactose values higher than 40 mg. per 100 cc. thirty to sixty minutes after ingestion of 40 gm. of galactose are suggestive of hepatic functional impairment. Similar findings have been reported in osteitis deformans (Paget's disease). The intravenous test may be of greater value (p. 30). Ninety-seven per cent of patients with cirrhosis, 81 per cent of those with hepatitis and only 18 per cent of those with obstructive jaundice of less than six months' duration were found to have a blood galactose concentration greater than 20 mg. per 100 cc. (normal, zero) seventy-five minutes after intravenous injection of 1 cc. of a 50 per cent solution of galactose per kilogram of body weight. This test yields normal results in patients with uncomplicated hyperthyroidism.

Blood Lactic Acid. A portion of the lactic acid that results from the breakdown of glycogen in the muscles is carried in the blood to the liver, where that fraction of it which escapes immediate combustion is converted into glycogen. Normal values for lactic acid in venous blood range from 6 to 20 mg. per 100 cc. In the presence of hepatic functional impairment, with disturbance in the glycogen-forming mechanism of the liver, it can be readily understood how a diminished capacity on the part of the liver for removing lactic acid from the blood and reconverting it into glycogen may result in an increase in the concentration of lactic acid in the circulating blood. Such an increase has been reported by several observers, values as high as 46 mg. per 100 cc. having been observed in some cases of extensive, diffuse hepatic parenchymal disease such as occurs in advanced cirrhosis, arsphenamine hepatitis, and other varieties of acute diffuse necrosis of the liver. However, normal findings are frequently obtained in all types of hepatic disease, and the fact that increased values usually appear only in the terminal stages of these conditions renders the determination of the lactic acid content of the blood of little value from a practical standpoint in the estimation of the state of hepatic function.

It has been found that in some cases of hepatic disease, intravenously injected lactic acid or sodium lactate disappears from the blood more slowly than in the case of normal subjects and, consequently, the "lactic acid tolerance test" has been suggested as a means of studying the glycogenic function of the liver.

The following procedure may be employed: A control sample of blood is collected in fluoride before breakfast, after a fasting period of twelve hours. The subject then receives, intravenously, 75 mg. per kilogram of body weight of sodium-*d*-lactate, in 10 to 14 per cent solution. Blood samples are collected in fluoride thirty minutes after the injection and blood lactic acid determinations are made. It has been found that the increase in blood lactic acid above the resting control value ranges from about 15 to 42 mg. per 100 cc., the peak being reached at the end of five minutes. Subsequently, the lactic acid concentration of the blood falls rapidly, the major drop occurring within twenty minutes, returning approximately to the control level within thirty minutes. It has been found that in the presence of acute diffuse hepatic parenchymal damage there is a definite delay in the utilization of intravenously injected lactic acid, due presumably to the difficulty of conversion of the available lactate into glycogen by the damaged liver. An elevation of 5 mg. or more above the control blood lactic acid value at the end of thirty minutes is regarded as evidence of liver damage. Preliminary observations suggest that essentially normal findings are obtained in patients with jaundice due to extrahepatic biliary tract obstruction. This procedure may therefore prove to be of value in differentiating between obstructive and hepatocellular types of jaundice.

Pyruvic acid may be increased in the blood and cerebrospinal fluid in advanced hepatic insufficiency, e.g., in decompensated cirrhosis. This has been attributed to inadequate utilization of pyruvate by the liver (p. 57).

PROTEIN METABOLISM

The liver plays an important part in normal protein metabolism. Its chie function in this connection, in man, appears to be the deamination and transamination of amino acids, urea formation, and the formation of certain plasma proteins (p. 144).

Amino Acids (p. 182). Under normal conditions the liver is the main site of deamination of amino acids. However, because of the presence of an enormous factor of safety, 90 per cent or more of this organ must be removed before this function is significantly impaired. Complete hepatectomy results in a steadily progressive increase in the concentration of amino acids in the blood. Because of this large functional reserve, the amino acid content of blood and urine is usually within normal limits in the more common diseases of the liver. Elevated values may however be observed in conditions associated with acute widespread degenerative lesions of the liver. This occurs characteristically in acute yellow atrophy, phosphorus, chloroform and carbon tetrachloride poisoning, arsphenamine hepatitis, cinchophen poisoning and in some cases of eclampsia. In these conditions the amino acid nitrogen content of the blood may be 10 to 15 mg. per 100 cc., occasionally rising to as high as 30 mg. In one reported case, values above 200 mg. were observed just before death. The increase in amino acids in degenerative lesions of the liver is probably due partly to inefficient deamination of amino acids and partly to extensive autolysis of hepatic tissue. The increased concentration of amino acids in the blood is associated with a corresponding increase in the amino acid content of the urine. Under such circumstances, amino acids may be present in the urine in large quantities. Normally free amino acids exist in the urine in small amounts (p. 151). Consequently, the positive identification of spontaneously occurring leucine and tyrosine crystals in the urine has been assumed to be strongly indicative of the presence of an extensive hepatic degenerative process such as occurs in acute yellow atrophy of the liver.

The abnormal amount of amino acids excreted in the urine of patients with acute hepatic necrosis originates chiefly from autolysed liver tissue. In some cases aminoaciduria may occur in the absence of apparent disturbance in the metabolism of amino acids. Continuous, massive tyrosinuria (0.9–2 gm. daily) has been observed only in cases of acute yellow atrophy of the liver with a rapid and fulminating course. Transitory, minimal and moderate tyrosinuria has been observed in cases of subacute atrophy of the liver, in degenerating neoplasm of the liver, in toxic hepatic degeneration and, rarely, in patients with obstructive jaundice of long standing due to stone. Tyrosinuria does not occur in patients with uncomplicated inflammatory lesions of the bile passages but is found occasionally in the presence of extrahepatic autolytic foci, such as degenerating tumors of the lung or extensive sloughs of the skin. During the phase of recovery from degeneration of the liver, tyrosine vanishes from the urine, but with a fresh exacerbation of the hepatocellular lesion tyrosine reappears in the urine. Negative findings may be obtained in the stage of repair, as in nodular cirrhosis, or in the terminal stages of subacute yellow atrophy with a critical reduction in the amount of functioning parenchyma of the liver. Increasing tyrosinuria is assumed to indicate a rapidly progressive degenerative process in the hepatic parenchyma. Although this finding appears to be of distinct prognostic value, the absence of tyrosinuria in such cases does not necessarily warrant an optimistic prognosis.

Excessive aminoaciduria occurs also in patients with subacute and chronic cirrhosis of the liver (about 20% of cases) (p. 182), more frequently in those with jaundice. Although the urinary amino acid excretion tends to increase with progression of the disease, and to decrease with clinical improvement, this factor is not generally of diagnostic or prognostic value. The rather consistent occurrence of aminoaciduria in hepatolenticular degeneration (Wilson's disease) is referred to elsewhere (p. 182). It is not necessarily associated with an increase in blood amino acids in this condition, being due apparently to inadequate reabsorption from the glomerular filtrate by the renal tubular epithelium. The relation of this abnormality to the lowered level of copper in the plasma (ceruloplasmin) and its increased urinary excretion, also apparently characteristic manifestations of this disease, is not known.

Urea. Urea is formed in the liver from ammonia derived from amino acids during the process of deamination (p. 136). The liver is the only important site of urea formation. The fact that the production of urea in the body ceases following total hepatectomy would naturally lead to the assumption that hepatic insufficiency should be associated with a decrease in the urea content of blood and urine. In general, the statements made with regard to the relationship of hepatic disease to the metabolism of amino acids apply equally to urea. Deamination and urea formation are closely related phenomena; since the quantity of urea formed by the liver appears to be determined by the efficiency of the mechanism of deamination, it naturally follows that diminution in the concentration of urea in the blood and urine occurs only in those conditions in which the blood and urinary amino acid nitrogen is increased.

In the majority of normal individuals the blood urea nitrogen concentration ranges from 12 to 15 mg. per 100 cc. However, values of 9 to 18 mg. per 100 cc. cannot be considered abnormal. The hepatic lesions which produce subnormal values are those which interfere with the process of deamination, and include, as stated above, such conditions as acute yellow atrophy of the liver, phosphorus, arsphenamine, chloroform and carbon tetrachloride poisoning and, at times, eclampsia. In one very severe case of acute toxic necrosis of the liver the blood urea nitrogen was zero shortly before death. Values below 6 mg. per 100 cc. are quite unusual. In conditions such as cirrhosis, syphilis, malignancy, hepatitis and obstructive jaundice, the blood urea nitrogen is usually within normal limits. Subnormal values occurring in biliary tract disease, particularly in obstructive jaundice, are of serious prognostic significance, since they usually indicate a superimposed lesion of the hepatic parenchyma. The normal ratio of blood urea nitrogen to amino acid nitrogen is approximately 2 to 1. A decrease in this ratio, indicating a decrease in the deaminizing capacity of the liver, is usually significant of advanced grades of hepatic insufficiency. Under such circumstances the total nonprotein nitrogen content of the blood and urine is not necessarily altered. Determination of the nitrogen partition characteristically reveals a decrease in the proportion of nitrogen present in the form of urea and a corresponding increase in amino acid and ammonia nitrogen. Because these changes occur only in far advanced hepatic disease they are of serious prognostic significance but are of no diagnostic value.

Uric Acid. There is evidence which suggests that the liver plays an important part in the destruction of uric acid in the dog. Following complete hepatectomy large amounts of uric acid are excreted in the urine in this species. On this basis it has been suggested that determination of the uric acid content of blood and urine may be used as a test of hepatic functional efficiency. However, there is no satisfactory evidence that uric acid is destroyed in man (except by intestinal bacteria), and such studies have proved to be of no clinical value in this connection. Although a slight increase in blood uric acid may be observed in some patients with biliary tract disease, cirrhosis, and obstructive jaundice, such findings are not common. In fact, low values have been reported in severe cases of acute necrosis of the liver.

Ammonia. The blood ammonia concentration, normally very low (<1 mg./100 cc.), has been found to be elevated (to 10 mg./100 cc.), but not consistently, in severe hepatic functional impairment. It has been suggested that this may play a significant role in the development of hepatic coma. Increase in blood ammonia, with precipitation of coma, has been observed in certain cases of cirrhosis following oral administration of ammonium exchange resins for the purpose of controlling ascites and edema.

Plasma Proteins. (*a*) FIBRINOGEN. Fibrinogen is formed entirely in the liver. Following total hepatectomy the plasma fibrinogen falls progressively, the body being apparently unable to regenerate this substance in the absence of the liver. Clinically, remarkable variations may be observed in the fibrinogen content of blood plasma in liver disease. Analysis of the reported observations appears to indicate that mild liver injury is associated with normal (200–400 mg. per 100 cc.) or slightly increased values. In the presence of severe liver damage the fibrinogen content of the blood plasma may fall to extremely low levels, figures as low as 50 mg. per 100 cc. having been observed in cirrhosis. As a general rule, the determination of the plasma fibrinogen content is of little value in the early diagnosis of hepatic disease, but low values are usually indicative of extensive liver damage.

(*b*) ALBUMIN. The liver is the organ chiefly if not entirely responsible for the formation of the plasma albumin and at least 80 per cent of the plasma globulins, i.e., the α- and β-globulins (p. 144). It would therefore be anticipated that hepatic functional impairment might result in decrease in the plasma albumin concentration. This expectation is borne out in practice.

Hypoalbuminemia may occur in chronic liver diseases, such as portal cirrhosis, chronic passive congestion, and carcinoma. It may be quite marked in cirrhosis, contributing to, but not entirely responsible for the development of ascites and generalized edema in that condition (p. 315). It is a rather constant feature of "kwashiorkor," a form of hepatic disease occurring in children in tropical countries, presumably due primarily to protein malnutrition. The low values in cancer of the liver may be due to the frequently present cirrhosis, or to the associated inanition. In congestive heart failure, the decrease in albumin, although it may be dependent in part on impaired liver function (p. 157), is contributed to also by other factors, including plasma dilution (p. 158) and gastrointestinal factors (p. 157).

Hypoalbuminemia is demonstrable in some cases of acute infectious and serum hepatitis and, more consistently, in acute and subacute hepatic necrosis (e.g., carbon-tetrachloride and chloroform poisoning). Studies in human volunteers infected with the virus of infectious hepatitis have revealed a rather consistent fall in albumin within the first few days, reaching a minimum at about 8 to 10 days, and then usually rising gradually to attain a normal level in 5 to 6 weeks. In general, the degree and duration of the hypoalbuminemia paralleled the clinical severity of the hepatitis. As indicated above, decrease in plasma albumin is not observed so consistently in spontaneous forms of this disease.

Subnormal plasma albumin concentrations are found in 30 to 50 per cent of patients with extrahepatic biliary obstruction, e.g., carcinoma of the head of the pancreas and common duct stone. This may be due in some cases, particularly in cholelithiasis, in part at least to superimposed hepatocellular damage, and in others to imposed or voluntary diet restriction, digestive disturbances (e.g., in associated pancreatic duct obstruction or pancreatitis), or inanition (e.g., in malignancy).

(*c*) GLOBULINS. Certain generalizations may be made concerning changes in plasma globulins in hepatic disease (pp. 159–162). In those conditions characterized by hepatocellular damage, there is commonly an increase in globulin, usually largely in γ-globulin, but at times also in α- and β-globulins. This alteration in globulin pattern is usually, but not invariably, associated with a decrease in albumin. The underlying mechanisms are not clear in all instances, but certain possibilities may be indicated. Increase in α-globulins, which occurs in many conditions accompanied by fever, and inflammatory and degenerative processes (p. 159), may be related to these factors in infectious and other forms of hepatitis, and in hepatic necrosis and cirrhosis. Increase in β-globulins may be related to the abnormality in plasma lipids (lipoproteins, p. 112) that occurs in certain forms of liver disease. These globulin fractions are believed to be synthesized mainly in the liver. The increase in γ-globulins, which are believed to be formed largely in extrahepatic tissues (reticuloendothelial cells) (p. 159), and which represent the bulk of the circulating antibodies, is attributed to different causes by various investigators. In hepatitis due to infectious agents, e.g., viral hepatitis, it may represent in part the development of specific anti-virus antibodies. It has been suggested also that the γ-globulin increase may be due to the production of antibodies to altered, i.e., abnormal (foreign), liver-cell proteins resulting from hepatocellular damage induced by infectious and toxic chemical (e.g., carbon tetrachloride, chloroform, arsphenamine, etc.) agents. In the presence of liver damage, the impaired capacity of the liver for utilizing amino acids for synthesis of its cell proteins and plasma albumin may make available increased amounts of these amino acids for synthesis of γ-globulins by extrahepatic tissues.

Studies in human volunteers infected with the virus of infectious hepatitis indicate that the γ-globulins begin to increase in the plasma within the first three days, i.e., before the onset of jaundice, reach a maximum concentration in about eight to ten days, and remain at an elevated level for three to four months in the average case. This is accompanied by hypoalbuminemia, usually of briefer duration (p. 157), a plasma protein pattern observed frequently in a variety of acute infections. In spontaneously occurring viral hepatitis, abnormally high concentrations of γ-globulin are encountered in about 70 per cent of cases, and in β-globulin, usually elevations of lesser magnitude, in 30 to 50 per cent. An increase in α-globulins occurs in some cases; it has been suggested that this may be associated with a tendency toward progression to chronic hepatitis. Although the increase in γ-globulins is generally greater in more severe cases, this correlation is not consistent. However, persistence of this abnormality beyond the usual expectation ($3\frac{1}{2}$–$4\frac{1}{2}$ months) may reflect progression of the acute hepatitis to cirrhosis. Residual abnormalities in globulin pattern may persist occasionally for one to three years, without other evidence of disease and with no indication of permanent liver damage.

Similar changes, usually more marked, occur in cases of acute and subacute hepatic necrosis, i.e., idiopathic, "kwashiorkor" (dietary?), or due to hepatotoxic agents, such as carbon tetrachloride, chloroform, etc. γ-Globulin values over 8 gm./100 cc. (average normal, 0.9 gm.) have been observed in conditions in this category, and also in patients with postnecrotic cirrhosis. In other types of cirrhosis, globulin values up to 6.5 gm./100 cc. are not unusual, the increase being largely in the γ-globulin fraction. In biliary cirrhosis, increase in α- and/or β-globulins may be associated with the increase in plasma lipids (lipoproteins) that occurs frequently in this condition (p. 112). The γ-globulins may be increased in 40 to 60 per cent of cases of carcinoma of the liver. Interpretation of the significance of this observation is difficult because of the frequent coexistence of cirrhosis.

In general, increase in γ-globulins is a sensitive reflection of diffuse hepatic disease, inflammatory (hepatitis) or degenerative (necrosis), usually occurring early, and usually persisting after disappearance of subjective and other objective manifestations. Extreme hyperglobulinemia may indicate an unfavorable prognosis, but not invariably. As indicated above, undue persistence of this abnormality in patients with acute hepatitis or necrosis may indicate progression to chronic disease (chronic hepatitis, portal cirrhosis, cholangiolitic biliary cirrhosis). It may be of significance in connection with the plasma protein changes in cirrhosis that liver ischemia induced by surgical restriction of the hepatic circulation has been found to be followed by a decrease in albumin and an increase in β- and γ-globulins. During actively progressing stages of the acute diseases, and also in cirrhosis, the increase in globulins is commonly accompanied by decrease in albumin (p. 157). Whereas these findings are by no means restricted to hepatic disease, occurring also in a variety of acute and chronic infections, the nephrotic syndrome, multiple myeloma, sarcoidosis, etc. (p. 161 ff.), they often aid in differentiating between hepatocellular and uncomplicated extrahepatic obstructive jaundice. Whereas a decrease in plasma albumin, usually of comparatively moderate degree (3.0–3.5 gm./100 cc.) is not uncommon in obstructive types of jaundice (p. 506), only a small proportion (15–25%) of such cases with hypoalbuminemia also exhibit hyperglobulinemia, in the absence of other evidence of superimposed hepatocellular damage.

(*d*) TOTAL PROTEIN. Because of the frequent occurrence of simultaneous changes in albumin and globulin in opposite directions in hepatic diseases, as indicated above, the total plasma protein concentration may be within normal limits, subnormal, or elevated, depending upon the relative magnitude of these changes. Normal values may be obtained in the early stages of infectious hepatitis, due to an equal decline in albumin and rise in globulins, with elevated values later, due to the frequent persistence of hyperglobulinemia for some weeks after the albumin has returned to a normal level. In cirrhosis, values of 5 to 6 grams of globulin and 1 to 3 grams of albumin per 100 cc. are not unusual, the total protein concentration being within normal limits (6–8 gm./100 cc).

It must be remembered that the range of normal variation in total globulin concentration, i.e., 2.1 to 4.2 gm./100 cc., permits a relatively large increase in a single component, e.g., γ-globulin (average normal, 0.9 gm./100 cc.) without a demonstrable significant increase in total globulins above the upper limit of normal. Such abnormalities can be revealed by electrophoresis or zinc sulfate turbidity measurements, and by certain of the so-called globulin reactions, e.g., the thymol turbidity and flocculation tests, and the cephalin-cholesterol flocculation test.

The total protein may be quite low in the face of a 100 or 200 per cent increase in γ-globulins, if the albumin concentration is very low (e.g., 1–2 gm./100 cc.), as may be the case in cirrhosis or hepatic necrosis. However, rather high total protein values, e.g., over 10 gm./100 cc., may be found in these conditions, especially in severe cases, and particularly in subacute necrosis and postnecrotic cirrhosis, in which the decrement in albumin is markedly overcompensated for by the large increment in globulins (p. 162).

(*e*) GLOBULIN REACTIONS. The various procedures in this category and the mechanisms involved in the production of abnormal reactions are reviewed elsewhere. Those employed most widely in patients with hepatic and biliary tract disease are: (1) the cephalin-cholesterol flocculation test; (2) the thymol turbidity and flocculation tests; (3) the zinc sulfate turbidity test.

It should be emphasized that these are not tests of liver function in the strict sense, at least insofar as the production of abnormal reactions depends upon an increase in γ-globulin. This is a reflection of morphologic damage to liver cells (in hepatic disease) or of an immunologic response to a causative infectious agent. Hypoalbuminemia, and changes in α- and β-globulins, which may influence certain of these reactions, may result, directly or indirectly, from impairment of liver function. However, none of these phenomena is specifically indicative of liver disease, and abnormalities in these directions do not necessarily parallel the results obtained with true tests of liver function. In fact, their greatest practical value, i.e., in differentiating between hepatocellular and obstructive types of jaundice, arises out of the fact that, unlike true function tests, these reactions appear to reflect the presence and extent of morphological liver damage (in hepatic disease), although they are influenced also by coexisting extrahepatic processes.

Thymol Turbidity and Flocculation (p. 164). Although increase in γ-globulins may contribute to the production of abnormal reactions with this test, these, in contrast to cephalin-cholesterol flocculation, are comparatively unaffected by changes in plasma albumin and are enhanced by increases in lipid components, probably β-lipoproteins (β-globulins) (p. 112). It must be remembered in this connection that an abnormal thymol test may be obtained with hyperlipemic srum, e.g., in diabetes, and in normal subjects after a fat meal.,

In infectious ehepatitis, and, to a lesser extent, in acute necrosis of the liver there is a fair degree of correspondence between the increment in plasma globulins, as determined chemically, chiefly the euglobulin fraction (β- and γ-globulins), and the incidence of positive thymol reactions. However, the degree of positivity varies widely with samples of serum containing similar amounts of globulins obtained from different patients or at different stages of the disease in the same patient. This correspondence is usually not so consistent in cirrhosis.

Clinically, there is generally good qualitative agreement (70–90%) between the thymol and cephalin-cholesterol reactions (i.e., both normal or both abnormal) in hepatic and biliary tract disease, although there are frequently differences in the degree of abnormality. Many of the qualitative differences (one test normal, the other abnormal) are of a borderline nature, but some are striking. The incidence of abnormal thymol turbidity (5–12 units, occasionally 20, rarely 30–40 units) and flocculation is somewhat lower than that of abnormal cephalin-cholesterol flocculation in acute active stages of viral hepatitis, acute and subacute necrosis, and portal cirrhosis. The thymol tests are more often negative in malignant biliary obstruction and in carcinoma of the liver than is the cephalin-cholesterol flocculation test, an observation which may be of some diagnostic value. However, in these conditions, as well as in cirrhosis, instances are encountered of the reverse situation, i.e., positive thymol, negative cephalin-cholesterol tests. During convalescence from hepatitis, abnormal thymol reactions occur more commonly and persist longer than does abnormal cephalin-cholesterol flocculation.

The thymol turbidity test has certain practical advantages over the cephalin-cholesterol test: (1) rapidity (30 minutes, vs. 24–48 hours), (2) stability of reagents, and (3) more precise quantitation of results. However, the frequency with which discrepancies between these two reactions occur in common types of hepatic and biliary tract disease makes it undesirable to rely on the thymol tests alone in the study of patients with these disorders. Both should be performed simultaneously, a practice that is entirely feasible because of their technical simplicity and the small amounts of serum required. It should be recalled that, in acute hepatic diseases, significant thymol flocculation (30 hours) may occur in the absence of abnormally high turbidity values (p. 164). Consequently, the presence of flocculation should be investigated routinely, particularly if the turbidity test yields normal results.

In general, the statements made with regard to the relationship between severity of the liver lesion and changes in plasma globulin (p. 507) at different stages of acute hepatic diseases apply to abnormalities in these globulin reactions. Their prognostic significance is limited during acute phases of hepatic disease (hepatitis and necrosis), but undue persistence of abnormalities may suggest progression to cirrhosis. The value of the thymol tests in differential diagnosis is discussed elsewhere (p. 551).

Cephalin-Cholesterol Flocculation (p. 164). Flocculation of the cephalin-cholesterol emulsion with human serum under appropriate conditions appears to depend upon the existence of the following conditions:

(1) Increase in γ-globulin in such quantity that the serum albumin, which stabilizes the emulsion, is unable to inhibit the reaction.

(2) Decrease in serum albumin concentration below that capable of inhibiting the reaction.

(3) Decrease in flocculation-inhibiting properties of the serum albumin fraction due to its chemical modification.

Positive reactions may be obtained with serum of certain normal laboratory animals, including dogs and rabbits, and with normal human serum (*a*) after standing at refrigerator temperatures for several months, (*b*) after heating to 56° C. for thirty minutes, or (*c*) if the reaction mixture is exposed to light during the test period. A 3 or 4 plus flocculation is regarded as a positive (abnormal) result, 2 plus as questionable.

The clinical value of the cephalin-cholesterol flocculation test in the study of patients with hepatic disease arises out of the fact that the conditions required for the production of an abnormal reaction are present in a rather large proportion of cases of hepatocellular damage. This test is positive in: 80 to 85 per cent of cases of viral hepatitis, during the active stage; other forms of hepatitis, 20 to 25 per cent; acute and subacute hepatic necrosis, 80 to 90 per cent; portal cirrhosis, 70 to 75 per cent; extrahepatic biliary obstruction, 30 to 35 per cent (mainly calculous obstruction); cancer of the liver, 30 to 60 per cent (usually in patients with hyperbilirubinemia and cirrhosis); occasionally in chronic passive congestion. The usefulness of this procedure in differential diagnosis is discussed elsewhere (p. 551 ff.).

Zinc Sulfate Turbidity (p. 163). Results obtained with this procedure in subjects with hepatocellular damage or biliary tract disease in general parallel those obtained with other globulin reactions. This parallelism, however, is not observed consistently, inasmuch as the zinc sulfate turbidity test reflects quantitative changes in γ-globulins more specifically than do the thymol or cephalin-cholesterol tests, being relatively uninfluenced by changes in plasma albumin and lipids, which affect the latter procedures.

In acute hepatitis, elevated values (above 8 units) may appear earlier and persist longer than abnormalities in other globulin reactions. The incidence of abnormal zinc turbidity in hepatic and biliary tract diseases is approximately as follows: viral hepatitis, 70 to 80 per cent; toxic hepatitis, 50 to 60 per cent; cirrhosis, 80 to 90 per cent; carcinoma of the liver, 60 to 70 per cent; extrahepatic biliary obstruction, 25 to 30 per cent.

The chief clinical value of this procedure is as an aid in distinguishing between hepatocellular and extrahepatic obstructive types of jaundice (p. 551ff.) and, to a lesser degree, in determining progression of acute liver lesions to cirrhosis.

Other Globulin Reactions. Other procedures may be employed for studying plasma protein abnormalities in hepatic disease. These include the colloidal gold (p. 163), formol-gel (p. 163), Takata-Ara (p. 165), CO_2 saturation (p. 164), Weltmann (p. 165), and Bauer (p. 165) tests. In our experience, none of these possesses any advantages over the thymol, cephalin-cholesterol, and zinc sulfate tests, which are preferable for purposes of routine study of patients with jaundice.

Plasma Prothrombin. Prothrombin, a constituent of the blood plasma essential for normal coagulation of blood, is generally believed to be protein in nature, being associated with the pseudoglobulin fraction of the plasma proteins. It is formed in the liver, an adequate supply of vitamin K being necessary for its formation in normal amounts (p. 469).

The hemorrhagic tendency manifested by many patients with obstructive, as well as by some with hepatocellular jaundice and bile fistula, is dependent chiefly upon severe prothrombin deficiency. At least two factors are of significance in this connection, being necessary for the maintenance of a normal plasma prothrombin concentration: (*a*) adequate hepatocellular function and (*b*) absorption of an adequate amount of vitamin K from the intestine. Inasmuch as bile salts are necessary for proper absorption of vitamin K (p. 468), inadequate absorption, with consequent hypoprothrombinemia, may occur (1) when bile is absent from the intestine due to bile-duct obstruction or external bile fistula and (2) when there is deficient formation and excretion of bile salts by the liver. Hypoprothrombinemia may also occur in hepatic disease due to inability of the liver to utilize vitamin K satisfactorily for the formation of prothrombin (hepatitis, cirrhosis, acute or subacute hepatic necrosis, chronic passive congestion, fatty liver, extensive hepatic malignancy, infectious hepatitis, etc.). A drop in prothrombin also occurs frequently after operations on the biliary tract.

With the two-stage method for prothrombin determination, hypoprothrombinemia has been demonstrated in a large majority of patients with jaundice of either obstructive or hepatocellular origin. The one-stage method, which is simpler and more commonly employed clinically, reveals this deficiency in a smaller proportion of cases. No direct methods are available for accurate clinical determination of prothrombin, the indirect methods in common use representing assays of the thrombin-forming capacity of the plasma under controlled conditions.

There is apparently a wide margin of safety in the prothrombin factor, and the coagulation time as measured by usual methods may remain within normal limits until more than 80 per cent of the prothrombin of the blood is lost. When the concentration of prothrombin in the plasma falls below about 30 per cent of normal, the "prothrombin time" becomes longer than twenty seconds (upper limit of normal); with further reduction of the prothrombin concentration to less than 20 per cent of normal, the "prothrombin time" becomes markedly prolonged. When the latter is longer than forty seconds, indicating prothrombin concentration in the plasma of less than 10 per cent of normal, the existence of a hemorrhagic tendency may be expected. Experimentally, plasma prothrombin deficiency has been produced in animals by common-duct ligation, partial hepatectomy, biliary fistula, and the administration of hepatotoxic substances such as chloroform and carbon tetrachloride.

Since hypoprothrombinemia in uncomplicated common-duct obstruction (stone, stricture, tumor, pressure) is due to absence of bile salts in the intestine, with consequent inadequate absorption of vitamin K, a prompt increase should follow parenteral administration of the latter or its oral administration in conjunction with bile salts. On the other hand, hypoprothrombinemia in patients with hepatocellular damage is dependent largely upon inability of the liver cells to form prothrombin, and little or no increase should follow administration of vitamin K. These facts have been utilized in attempts to differentiate between obstructive and hepatocellular types of jaundice, the capacity for responding to vitamin K being interpreted as an index of the adequacy of hepatic cell function. In the presence of hypoprothrombinemia in a patient with jaundice, a significant increase following administration of vitamin K favors the diagnosis of obstructive jaundice, whereas the absence of such increase suggests the presence of impaired hepatocellular function (p. 469). The criteria for a satisfactory response vary considerably. Some regard a failure to obtain a rise of more than 10 per cent in twenty-four hours after intramuscular injection of a vitamin K preparation (2-methyl-1,4-naphthoquinone) as indicative of hepatocellular damage. With the oral administration of 8 mg. of this preparation (menadione) plus 2.5 gm. of bile salts or, preferably, parenteral injection of 10 mg. of tetrasodium 2-methyl-1,4-naphthoquinone diphosphoric acid ester (Synkovite-Roche), a significant rise of prothrombin may be expected within twenty-four hours in uncomplicated obstructive jaundice and little or no rise in hepatocellular jaundice.

There is a difference of opinion regarding the value of this procedure as an index of hepatic function and in differentiating between obstructive and hepatocellular jaundice. As stated previously, normal values for plasma prothrombin may be obtained (before giving vitamin K) in a considerable number of patients with obstructive or, more frequently, hepatocellular jaundice, especially when the one-stage method is used. Dogs poisoned with carbon tetrachloride exhibit first an increase in serum bilirubin and bromsulfalein retention and later an increase in serum phosphatase, while the plasma prothrombin and galactose tolerance change last or remain normal. Consequently, normal findings may be misleading. Subnormal values are more significant, but it must be remembered that hypoprothrombinemia may occur in conditions other than diseases of the liver and biliary tract (p. 469). Failure of the plasma prothrombin to respond satisfactorily to vitamin K administration is much more significant of impairment of hepatic function. However, the production of a satisfactory rise does not exclude the possibility of the existence of even considerable hepatocellular damage. The usefulness of this and other procedures in differentiating between obstructive and hepatocellular jaundice is discussed elsewhere (p. 551 ff.).

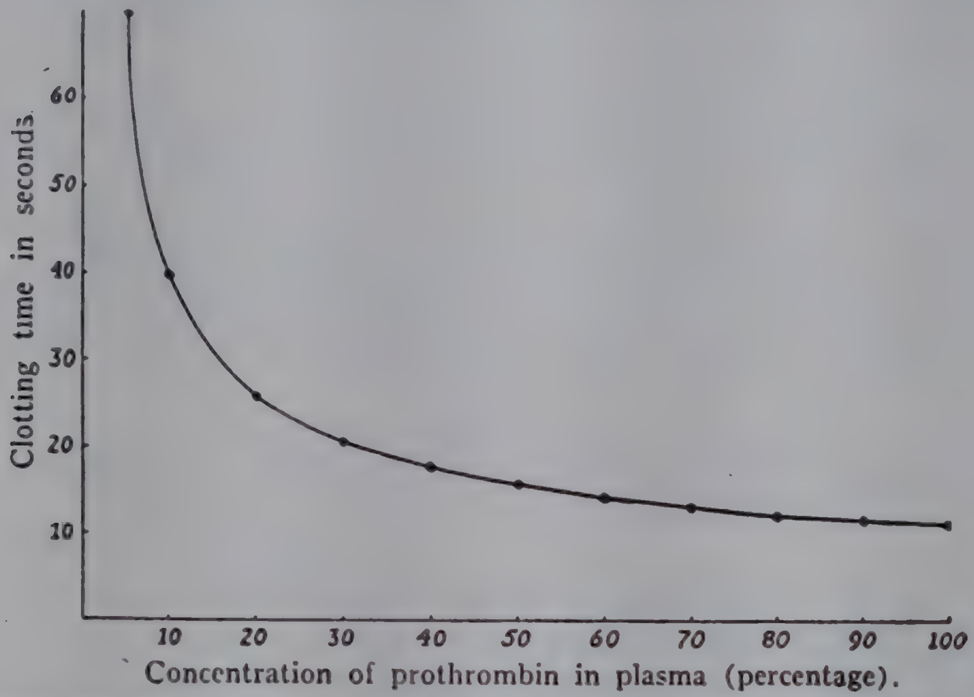

Fig. 51. Relation of clotting time of recalcified plasma (containing an excess of thromboplastin) to concentration of prothrombin. (After Quick.)

LIPID METABOLISM

The importance of bile in the absorption of fats from the intestine has been recognized for some time, and has been considered in detail elsewhere (p. 86). Its action in this connection may be summarized as follows: (*a*) activation of pancreatic lipase; (*b*) the bile salts aid in the emulsification and, consequently, the digestion of fat; (*c*) the bile salts form soluble addition compounds with fat or fat-soluble substances.

Fat in Feces (p. 96). Obstructive jaundice is usually accompanied by the presence of excessive quantities of fat in the feces (total fat, soap fat, free fatty acids). Similar findings may be obtained in infectious hepatitis, cirrhosis of the liver, toxic hepatitis and acute yellow atrophy of the liver. These changes have been attributed generally to decreased absorption of the products of fat digestion, a phenomenon which would naturally be expected to occur when bile is not present in the bowel in adequate amount. However, in view of present knowledge (p. 96) that fecal lipids consist of fatty material secreted or excreted into the bowel as well as unabsorbed portions of ingested lipids, this interpretation is open to question. Recent studies indicate that the absence of bile from the intestine causes but little impairment of fat absorption, 65 to 70 per cent of fatty acids of the diet being absorbed in patients and animals with bile fistula (external). It would appear, therefore, that, as under normal conditions, fecal fat in the absence of bile has, to a large extent, an endogenous origin. Moreover, since the excreted endogenous fat is in the form of neutral fat and fatty acids, it is obviously impossible to interpret alterations in the relative proportions of these substances in the feces in terms of altered digestion and absorption of the ingested fat.

Plasma Cholesterol. The alterations that may occur in plasma cholesterol concentration and partition in patients with hepatic or biliary tract disease have been considered elsewhere (pp. 116, 120). The significance of these changes may be summarized as follows:

(*a*) Hypercholesterolemia is found in the majority of patients with uncomplicated extrahepatic obstructive jaundice and occasionally in patients with cholelithiasis without obstruction of the common bile duct. The underlying mechanism is not known, although there is evidence which suggests that the increase may be in some way related to and dependent upon the increase in bile acids in the plasma which occurs simultaneously under these circumstances (p. 116). Although both free and ester fractions usually participate in this increase, the former usually rises disproportionately, with consequent decrease in the ester: total cholesterol ratio (in about 50 per cent of cases). In general, both total and ester values tend to be lower in neoplastic than in benign (calculous) obstruction.

Table 36. Partition of Fat in Feces (Fowweather)

	NORMAL* (average)	OBSTRUCTIVE* JAUNDICE
Total fat	17.5	50.9
Soap fat	4.6	26.8
Free fatty acid	5.6	18.1
Neutral fat	7.3	6.0

* Expressed as percentage of dry matter.

(*b*) Hypercholesterolemia occurs in certain forms of hepatic disease in the absence of extrahepatic biliary obstruction, due presumably to obstruction within the intrahepatic duct system. This is observed characteristically in cholangiolitic biliary cirrhosis (p. 116), and also in certain types of toxic hepatitis, particularly that due to arsphenamine.

Release of biliary obstruction is usually followed promptly by a return of the plasma cholesterol concentration to normal levels. Of the utmost importance from a diagnostic and prognostic standpoint is the fact that a similar fall may also occur simultaneously with manifestations of cachexia, terminal cholemia, infection, superimposed hepatocellular damage and other complications, even though the biliary obstruction persists. Under such circumstances, as is indicated below, the fall in plasma cholesterol occurs principally in the ester fraction. Repeated determination of the plasma cholesterol concentration and partition in patients with common duct obstruction is consequently of value in prognosis and treatment.

(*c*) Hypercholesterolemia occurs occasionally in patients with mild infectious hepatitis and cirrhosis, but much less frequently than in obstructive jaundice. When it occurs, the increase is usually mainly in the free cholesterol fraction, the ester cholesterol-free cholesterol ratio being diminished. Normal values for total cholesterol are the rule in infectious hepatitis (except in very severe cases), but the proportion of esters is frequently reduced. Except in the terminal stages, plasma cholesterol concentration and partition are usually normal in uncomplicated portal cirrhosis. Hypocholesterolemia is the rule in advanced forms of this condition, the decrease occurring chiefly in the ester fraction, as indicated below.

(*d*) Conditions accompanied by severe hepatocellular damage are frequently associated with a diminished proportion of cholesterol esters in the plasma (normally about 60–80% of the total), constituting the so-called "Estersturz." This may not be but usually is associated with a diminution in total cholesterol concentration. Among the conditions in which this phenomenon has been observed are acute and subacute hepatic necrosis, phosphorus and chloroform poisoning, and yellow fever. In these conditions the plasma cholesterol may be as low as 70 to 100 mg. per 100 cc. (normal 140–250 mg.), 0 to 50 per cent being in the ester form (normal 80–200 mg. per 100 cc., constituting 60–80 per cent of the total). In such cases, the degree of hypocholesterolemia and the fall in the ester fraction are often in inverse ratio to the degree of hyperbilirubinemia. It would appear that the degree of diminution in the cholesterol ester fraction in the plasma may be regarded as an indication of the extent of hepatocellular damage in these conditions. This phenomenon also is present when such damage occurs as a complication in patients with extrahepatic obstructive jaundice, a fall in the ester fraction often occurring in the presence of an initially elevated total choles-teral concentration.

The chief practical significance of this observation lies in the fact that it offers some assistance in differentiating hepatocellular jaundice from simple obstructive jaundice, in which hypercholesterolemia is the rule. Study of the relationship between the degree of bilirubinemia and the changes in plasma cholesterol may be of value in this connection. In jaundice occurring in acute degeneration of the liver, the blood cholesterol does not rise with the bilirubin but usually remains normal or subnormal. This divergence between the degree of blood cholesterol and blood bilirubin elevation in most cases of hepatocellular jaundice contrasts sharply with the parallelism between the hyperbilirubinemia and hypercholesterolemia in obstructive jaundice. The cholesterol ester is usually lowered in acute degeneration of the liver and mirrors the severity of the damage. When this phenomenon is observed in patients with common duct obstruction it should be regarded as of serious prognostic significance, particularly if it progresses and if the total cholesterol concentration also falls.

The liver is apparently the site of formation of the plasma cholesterol as well as the chief site of its esterification (p. 100). Accordingly, severe impairment of hepatic function would be expected to result in a fall in both total and ester cholesterol in the plasma. Theoretically, and frequently actually, these changes may aid in distinguishing between hepatocellular and extrahepatic obstructive types of jaundice, particularly if interpreted in conjunction with simultaneous changes in serum bilirubin concentration, as indicated above. However, their practical value in this connection is limited by the facts that normal values are not unusual in patients with common duct obstruction, and hypercholesterolemia is not unusual in certain forms of primary intrahepatic disease, e.g., in post-arsphenamine hepatitis, cholangiolitic biliary cirrhosis, and, occasionally, infectious hepatitis and portal cirrhosis. These facts must be borne in mind whenever data on plasma cholesterol are employed for this purpose.

PIGMENT METABOLISM—JAUNDICE

Bilirubin, the chief pigment of human bile, is derived from hemoglobin. It has been rather definitely established that the cells of the reticulo-endothelial system, especially those present in the bone marrow, spleen and liver (Kupffer cells), are concerned with the metabolism and formation of bile pigment. Although the hypothesis that the hepatic polygonal cells may play a part in the formation of bilirubin has not been entirely abandoned, it is generally agreed that their chief function in this connection is the removal of bilirubin from the blood stream and its excretion in the bile. The stages of practical importance in the degradation of hemoglobin are outlined in Figure 52.

The hemoglobin molecule contains three distinct components: (1) a protein, globin, (2) iron, and (3) a porphyrin, which is a cyclic structure composed of four pyrrole units (p. 203). The iron-porphyrin compound is termed "heme." When hemoglobin undergoes degradation, the liberated iron and globin are reutilized, but the porphyrin residue is not. Its cyclic structure is broken, and, after the iron has been removed, it is converted to an open-chain green pigment, known as biliverdin. This undergoes reduction to a yellow-orange pigment, bilirubin, which leaves the reticuloendothelial cell and enters the blood plasma firmly bound to an α-globulin. In this form it is carried to the liver, where it is separated from its protein carrier by either the Kupffer or polygonal cells. The latter excrete the free bilirubin into the bile canaliculi, from which it passes through the duct system into the duodenum.

About 7 to 8 gm. of hemoglobin are liberated daily as a result of normal erythrocyte destruction. The liver excretes 200 to 370 mg. of bilirubin daily in the bile, representing about 4 per cent by weight of the hemoglobin from which it is derived. In the intestine, this is reduced to mesobilirubinogen and stercobilinogen by the action of bacteria. These may be loosely collectively referred to as "bilinogens," or "urobilinogen." A fraction of the bilinogens is reabsorbed into the portal circulation and is carried to the liver (enterohepatic circulation); the major portion of this fraction is removed from the portal blood by the hepatic cells and is perhaps reconverted into bilirubin or a hypothetical pigment complex, the remainder escaping into the general circulation to be excreted in the urine (0–4 mg. daily). The portion which escapes reabsorption from the intestine is oxidized to urobilin and stercobilin (the "bilins"), which are the normal pigments of the feces (40–280 mg. daily, usually 100–250 mg.). There is some question as to whether any bilirubin is absorbed from the intestine; none is present normally in the feces of adults. It is present in the feces of normal infants, 1 to

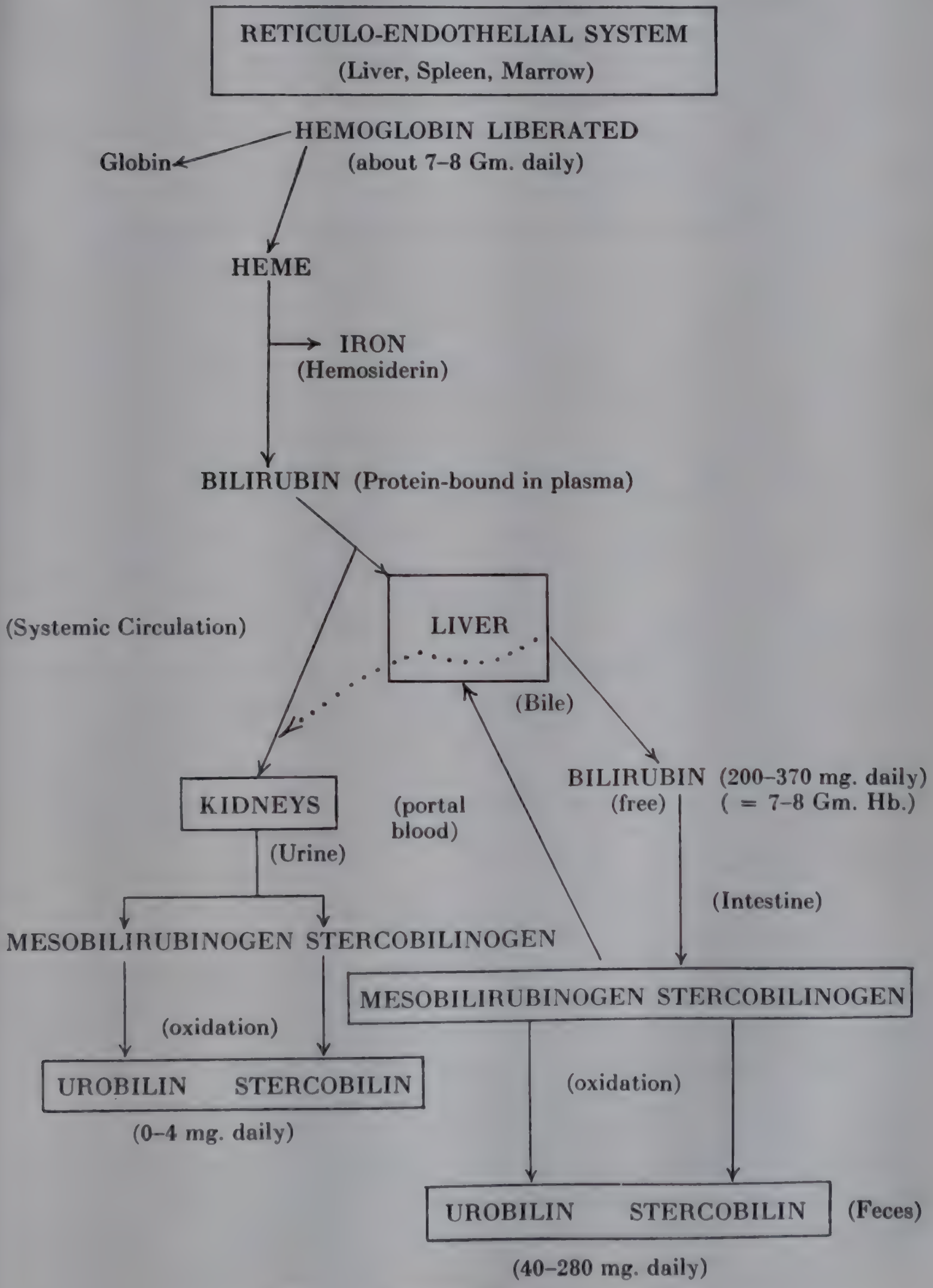
RETICULO-ENDOTHELIAL SYSTEM
(Liver, Spleen, Marrow)
HEMOGLOBIN LIBERATED
(about 7–8 Gm. daily)
Globin
HEME
IRON
(Hemosiderin)
BILIRUBIN (Protein-bound in plasma)
(Systemic Circulation)
LIVER
(Bile)
KIDNEYS
(portal blood)
BILIRUBIN (200–370 mg. daily)
(free)
(= 7–8 Gm. Hb.)
(Urine)
(Intestine)
MESOBILIRUBINOGEN STERCOBILINOGEN
MESOBILIRUBINOGEN STERCOBILINOGEN
(oxidation)
UROBILIN STERCOBILIN
(0–4 mg. daily)
(oxidation)
UROBILIN STERCOBILIN
(Feces)
(40–280 mg. daily)

Fig. 52. Catabolism of hemoglobin.

25 mg. (av. 8.6 mg.) daily in the first five days of life, 0 to 15 mg. (av. 5.7 mg.) daily during the second five days, decreasing gradually thereafter, disappearing in 8 to 75 days after birth. Fecal excretion of urobilinogen in infants ranges from 0 to 1.0 mg. daily during the first two weeks of life, increasing to an average of 25 to 50 mg. between five and ten years of age. Inasmuch as the molecular weights of the bilinogens and bilins are approximately the same as that of bilirubin, all being about 4 per cent of that of hemoglobin, the approximate hemoglobin wastage may be determined indirectly by determining the quantity of these pigments excreted in the urine and feces.

Porphyrins are red pigments with a pyrrole structure, which are important components of hemoglobin, myoglobin, cytochrome, and catalase. Their chemistry and metabolism are considered elsewhere (p. 203 ff.), and it is necessary here only to indicate certain features of importance in relation to hepatic function. There are four isomeric etioporphyrins, designated Types I, II, III and IV, but these are of practical importance only as reference types, the naturally occurring porphyrins corresponding to Types I and III. The production of these substances parallels hematopoietic activity and hemoglobin synthesis, Type III being formed in large amounts and utilized for the formation of hemoglobin and other respiratory factors, which are compounds of Type III porphyrin. Small amounts of coproporphyrin Type I are formed as a by-product in normal hematopoiesis and, perhaps with small amounts of coproporphyrin Type III not utilized in hemoglobin synthesis, are excreted, largely by the liver in the bile and eventually in the feces and urine. Under normal conditions, the daily excretion of coproporphyrin is about 60 to 280 (av. 160) micrograms in the urine and 300 to 1100 micrograms in the feces, the normal urine: feces ratio being about 1 : 5. About 60 to 80 per cent of the urinary coproporphyrin and 70 to 90 per cent of the fecal coproporphyrin is in the form of the Type I isomer, the remainder being Type III.

Clinical investigation of the state of liver and biliary tract function with regard to the metabolism of bilirubin and its derivatives includes studies of (*a*) serum bilirubin, (*b*) bilirubin tolerance, (*c*) urine bilirubin, (*d*) excretion of urobilinogen and related products in the urine and feces and (*e*) excretion of porphyrin in the urine and feces.

Serum Bilirubin

Qualitative van den Bergh Reaction. The van den Bergh test depends upon the fact that bilirubin combines with diazobenzosulfochloride (Ehrlich's diazo reagent) in alcoholic solution to form acetophenolazorubin. This reaction, requiring the addition of alcohol to the serum-reagent mixture, was termed the "indirect" reaction and is a specific test for bilirubin. The direct mixture of serum and reagent in aqueous-acid solution constitutes the "direct" reaction. The bilirubin of normal serum and that resulting from normal degradation of hemoglobin ("hemolytic" bilirubin) give only the indirect reaction. The bilirubin of bile gives both indirect and direct reactions. Consequently, the hypothesis has been proposed that some change occurs in the bilirubin molecule or in its mode of combination during its passage through the hepatic cells from the blood to the bile. The production of a positive direct reaction in the blood serum implies the presence of bilirubin that has re-entered the circulation after having been passed out of the blood, through the hepatic cells, into the bile canaliculi (e.g., biliary obstruction or hepatocellular damage) (p. 525 ff.).

Certain differences have been described between so-called "direct" and "indirect" bilirubin: (1) direct bilirubin is more readily oxidized; (2) when the serum proteins are precipitated with alcohol, direct bilirubin is adsorbed on the precipitate more readily than indirect bilirubin; (3) when serum is shaken with chloroform, indirect bilirubin is dissolved and direct is not; (4) direct bilirubin is dialyzable while the indirect is not. The last observation has not received general confirmation. The exact basis for this difference in reaction is not known. In the past, considerable attention was directed to the possibility that an increase in the concentration of surface-active substances (bile salts, cholesterol, fatty acids) may be responsible for the production of the direct reaction, but this hypothesis is no longer tenable.

Although normal serum bilirubin does not give a direct qualitative van den Bergh reaction (no color development within thirty seconds), it contains a fraction which reacts in aqueous-acid solution (direct reaction) in one to ten minutes, the magnitude of which varies somewhat in normal serum and in the serum of patients with various types of jaundice (p. 524). This demonstration of the presence of a considerable but variable quantity of direct-reacting bilirubin in normal as well as abnormal serum simultaneously with a nondirect-reacting fraction suggests that the essential difference lies either in (*a*) the bilirubin molecule or (*b*) its mode of combination in the serum, rather than in other factors in the serum that catalyze or inhibit the direct reaction. There is considerable evidence that the differences in reactivity are dependent on structural rather than catalytic factors, and that all of the bilirubin in human plasma of high bilirubin content is bound to the plasma albumin and globulin. However, there is also evidence that the capacity of serum bilirubin for reacting with the van den Bergh reagent in aqueous acid is dependent, in part at least, on factors in the serum other than the bilirubin molecule or the nature of its combination with plasma proteins.

The view that the production of the direct qualitative reaction is dependent primarily upon an increase in concentration of bilirubin in the serum is not tenable. A negative direct reaction has been obtained with the serum of subjects with congenital hemolytic jaundice at bilirubin levels as high as 9 to 12 mg. per 100 cc. and positive direct reactions in obstructive and hepatocellular jaundice at levels as low as 0.6 mg. per 100 cc. Moreover, there is direct evidence that the liver is implicated in the phenomenon that allows the production of the direct reaction. It is well known that following ligation of the common bile duct the serum bilirubin gives a direct reaction. If the liver is removed at a time when the direct reaction is given by the serum, the quantity of direct-reacting bilirubin remains unchanged subsequently. However, additional bilirubin accumulates in the blood after hepatectomy, so that the total concentration continues to increase at the same rate as after ligation of the common duct, but the added bilirubin gives only the indirect reaction.

Certain reasonably acceptable hypotheses may be advanced on the basis of evidence now available. The major portion of the bilirubin present normally in the blood is firmly bound to an α-globulin, this protein-bilirubin complex ("hemobilirubin") giving only the indirect van den Bergh reaction. In the liver, the bilirubin is separated from its protein carrier, and is excreted in the bile in the "free" state ("cholebilirubin"). In this form it can couple readily with the diazo reagent in aqueous-acid solution, thereby giving the direct van den Bergh reaction. In the presence of biliary obstruction, or of extensive hepatocellular damage, with disruption of the continuity of the canalicular epithelium, this "free" bilirubin ("direct-reacting") may be reabsorbed into the hepatic lymphatics or sinusoids. Re-entering the blood stream, this bilirubin becomes bound to plasma albumin in a union not sufficiently firm to prevent its dissociation under the conditions of the direct van den Bergh reaction, which is positive under these circumstances (i.e., obstructive and hepatocellular jaundice). As indicated below, there is a small amount of direct-reacting bilirubin in normal plasma, insufficient, however, to produce an immediate direct qualitative van den Bergh reaction. The source of this component is not known. It may conceivably represent bilirubin that has "leaked back" into the blood stream, from the liver cells or bile canaliculi, via the hepatic lymphatics.

Several types of direct reaction have been described: (*a*) Immediate direct reaction, in which a red-violet color develops immediately and attains a maximum intensity in ten to thirty seconds, the depth of color being roughly proportional to the amount of bilirubin. (*b*) Biphasic direct reaction, in which a reddish color appears immediately and changes to a red-violet either rapidly (biphasic prompt) or slowly (biphasic delayed). (*c*) Delayed direct reaction, in which a reddish color appears in from one minute to several hours, gradually deepening and acquiring a violet hue. It is the consensus at present that any distinction between the immediate and biphasic reactions is of no practical consequence and that the delayed direct reaction has the same significance as a negative direct reaction. The appearance of the characteristic color in aqueous acid within thirty seconds constitutes a positive direct reaction and absence of color development in this time is reported as a negative direct reaction. The clinical significance of these reactions in the presence of hyperbilirubinemia is discussed elsewhere (pp. 526, 527). Suffice it to state here that for practical purposes it may be assumed that the bilirubin in serum that gives a negative direct reaction has not passed through the hepatic cells, and that if the direct reaction is positive, some bilirubin has been reabsorbed into the blood (directly or via lymphatics) from the bile canaliculi (obstruction to flow of bile or hepatocellular damage).

Serum Bilirubin Concentration. The serum bilirubin concentration is usually determined either by a quantitative procedure based upon the van den Bergh reaction or by the icterus index determination.

QUANTITATIVE VAN DEN BERGH PROCEDURE. The normal serum bilirubin concentration in adults is 0.1 to 1.0 mg. per 100 cc., being below 0.8 mg. in about 75 per cent of cases and below 0.5 mg. in about 50 per cent. Reported values of 1.0 to 2.8 mg. per 100 cc. in apparently normal subjects probably represent instances of latent "familial" jaundice. The serum bilirubin concentration increases in untrained subjects ascending to high altitudes (anoxemia and excessive blood destruction). A state of physiologic hyperbilirubinemia is present during the first several days of life (up to 2 mg. per 100 cc. at birth), increasing to a maximum at about the fourth day (up to 11 mg.) and falling subsequently during the following week (up to 10 mg. at nine days). This is due perhaps to a higher rate of blood destruction than in adults, accompanied probably by some degree of inadequacy of hepatic function in removing bilirubin from the blood stream.

Although the qualitative direct van den Bergh reaction is negative, it is possible to demonstrate that normal serum contains both nondirect-reacting and direct-reacting bilirubin. Quantitative determinations of direct-reacting bilirubin may be of value, especially in cases in which the total serum bilirubin is within normal limits. Quantitative readings of the color developed within one minute are assumed to represent the amount of prompt-direct-reacting bilirubin. Values up to 0.24 mg. per 100 cc. have been obtained in normal subjects. An increase correlates fairly well, although not exactly, with the appearance of a positive direct qualitative van den Bergh reaction. Occasionally, an increase occurs before the qualitative direct reaction becomes positive. An increase is at times observed in hepatic disease in the presence of normal total serum bilirubin concentrations. This may be due, in part at least, to the relatively wide range of normal serum bilirubin values (0.1–1.0 mg. per 100 cc.), which makes it impossible in any given case to determine whether values between 0.5 and 0.8 mg. are or are not normal for any particular subject.

ICTERUS INDEX. Determination of the icterus index consists merely in comparing the intensity of the yellow color of the blood serum or plasma with that of a standard 1 : 10,000 solution of potassium bichromate (icterus index of the standard = 1). The icterus index of normal serum or plasma is 4 to 6, i.e., the yellow color is 4 to 6 times as intense as that of the standard. This is obviously an entirely nonspecific method, but is useful clinically because of its simplicity and because the intensity of the yellow color of the serum is usually dependent upon the bilirubin concentration. However, apart from technical difficulties due to hemolysis and turbidity of the serum, which interfere with the color comparison, difficulties in interpretation may arise from the fact that occasionally substances other than bilirubin may impart a yellow color to the serum. The most important of these are xanthophyll and carotin or carotene. In the presence of carotenemia (p. 456) the icterus index is increased and does not, of course, reflect the bilirubin content of the serum. The quantitative van den Bergh reaction is superior to the icterus index determination for the purpose of estimating the serum bilirubin concentration. In certain cases of protracted jaundice, particularly of the extrahepatic obstructive variety, a significant amount of biliverdin may appear in the plasma ("biliverdin jaundice"; "verdinicterus"). Inasmuch as this pigment does not give the van den Bergh reaction, icterus index values are relatively higher than the values for bilirubin measured by this procedure. A similar situation is said to occur also during periods of receding jaundice.

number and rate of destruction of erythrocytes (hemogl
functional activity of reticuloendothelial cells (bilirubin
polygonal cell function (removal of bilirubin from the bl
bile canaliculi and bile ducts (normal flow of bile) and (
the hepatic cell lining of the bile canaliculi.

Total Serum Bilirubin. Serum bilirubin concentrat
100 cc. or icterus index values above 6 constitute a stat
In the majority of cases, bilirubin concentrations greater t
abnormally high. The degree of hyperbilirubinemia neces
clinical icterus varies in different types of jaundice. It h
that discoloration of the sclerae and the skin and pass
the urine occur more commonly in obstructive and hepa
lytic jaundice. This has been explained on the basis of t
structive jaundice the bilirubin is more readily diffusible (
than in hemolytic jaundice (nondirect-reacting bilirubi
in obstructive and hepatocellular jaundice, when an icte
serum bilirubin concentration of 1.6 mg. per 100 cc. i
some days, bilirubin diffuses through the capillaries an
Greater concentrations are usually necessary in hemolyti
tion of frank icterus. It was formerly believed that a s
between the serum bilirubin concentration and the appe
urine. Although this is usually the case in obstructive ja
so in all forms of hepatocellular jaundice, notably infe
bilirubinuria may occur early, at relatively low seru
matter is discussed in more detail elsewhere (p. 528).

Once clinical jaundice and bilirubinuria have occurr
invariably, persist for some time after the serum biliru
"threshold" level. During the period of developing ja
bilirubin concentrations between 0.8 and 1.6 mg. per
values between 6 and 15 represent a condition of latent
tectable by methods other than examination of the bloo
of bilirubinemia, the ratio between the icterus index
centration, in milligrams per 100 cc., is roughly 10 :
corresponding to a serum bilirubin of 0.6 mg., and o
1.5 mg. This relationship has been found not to be so

ive direct van den Bergh reaction.

ous; hemolytic; sickle-cell; paroxysmal hemoglobinuria;
drazine, sulfonamides, etc.); mismatched transfusion.
: Congestion: cardiac decompensation, especially with
ction.

ociated with anoxemia, resulting from
tic septicemia; malaria; blackwater fever.
solidation: lobar pneumonia.

Cells. Icterus neonatorum.

t's cirrhosis.

flux of bilirubin from canaliculi into blood because of
pile or necrosis of liver cells (disruption of continuity of
van den Bergh reaction.

ls, caused by

: chloroform; carbon tetrachloride; phosphorus; arsphena-
fonamides, etc.
: mushroom poisoning.
: yellow fever; Weil's disease; congenital syphilis; infec-
atitis, etc.
ained: idiopathic "acute yellow atrophy"; portal cirrhosis.
of I, A and B.

Ducts, due to
i, biliary and pancreatic; inflammation; parasites (fasci-
); neoplasms.
carring, due to chronic inflammation, injury (*e.g.*, post-
hilis;
tions, as congenital stricture or atresia;
s.

lammatory masses in liver (abscess, gumma, tuberculosis,
se);
masses, as echinococcus cyst;
as;
l adhesions;
s;
odes.

to a decrease in the rate of formation of bilirubin as a
te of destruction of red blood cells, or to the oc-

therefore indicated in all cases in which information is desired regarding the possible presence of hepatic functional impairment, biliary stasis or excessive hemolysis. The frequency of latent or frank jaundice in both calculous and noncalculous cholecystitis as well as in hepatic disease, congestive heart failure and a variety of primarily extrahepatic disease processes emphasizes the importance of the estimation of the degree of bilirubinemia in these conditions. Experience has demonstrated the fallacy of attempting to estimate variations in bilirubinemia in patients with jaundice by observing variations in color of the skin and conjunctivae.

Table 38. Classification of Jaundice

(After Rolleston and McNee)

I. *Hemolytic.* Excessive production of bilirubin. Negative direct van den Bergh reaction.

Examples: Congenital hemolytic jaundice; icterus neonatorum; erythroblastosis fetalis; familial jaundice; paroxysmal hemoglobinuria; pernicious anemia; splenic anemia; Cooley's anemia; Marchiafava-Micheli syndrome; extensive burns; sickle-cell anemia; cerebral hemorrhage; ruptured ectopic pregnancy or other intraperitoneal hemorrhage; mushroom poisoning; favism; malaria; blackwater fever; hemolytic transfusion reactions; increased hemolysis incident to infection with such agents as streptococci, staphylococci, pneumococci, Clostridium perfringens, Bartonella bacilliformis; poisoning with snake venoms. arseniuretted hydrogen, toluenediamine, phenylhydrazine, acetanilid, sulfonamides, dinitrobenzol, aniline, benzol and nitro compounds of phenol, etc.

II. *Hepatocellular.* Injury to liver cells. Decreased removal of bilirubin from the blood and, if severe damage, escape of bilirubin from canaliculi into blood, due to cell necrosis and disruption of continuity of canaliculi. Direct van den Bergh reaction positive after very early stage.

Examples: Acute and subacute hepatic necrosis; infectious hepatitis; hepatitis due to any infection, as syphilis, pneumonia, dysentery, Clostridium perfringens, tuberculosis, typhus, typhoid, streptococcus, staphylococcus, relapsing fever; malaria; Oroya fever; infectious mononucleosis; hepatic cirrhosis; yellow fever; Weil's disease; congestive heart failure; hyperthyroidism; diabetes mellitus; poisoning with arsphenamine, sulfonamides, mercury, lead, chloroform, carbon tetrachloride, phosphorus, tetrachlorethane, trinitrotoluene, cinchophen; x-ray over liver.

III. *Obstructive.* Obstruction to flow of bile, usually confined to extrahepatic duct obstruction. Reflux of bilirubin from canaliculi into lymphatics or directly into blood, due to increased pressure within or rupture of canaliculi. Positive direct van den Bergh reaction.

Examples: Calculi, biliary or pancreatic; cholangitis; neoplasm of pancreas, ducts, duodenum or lymph nodes; duodenitis and diverticulum of duodenum; parasites in ducts; stricture of ducts, congenital or acquired; adhesions, pancreatitis; pancreatic cysts; cysts, carcinoma or abscess of the liver; aneurysm of the hepatic artery or renal artery.

The most widely accepted classifications of jaundice, based on pathogenesis, are presented in Tables 37 and 38.

ONE-MINUTE, DIRECT-REACTING BILIRUBIN. Normal serum contains 0.00 to 0.24 mg./100 cc. of bilirubin which reacts with the van den Bergh reagent in aqueous-acid solution (i.e., direct reaction) within one minute (p. 521). This is referred to as "one-minute, direct-reacting bilirubin." Increased values may be obtained in hepatocellular damage, biliary obstruction, and pernicious anemia, but not in uncomplicated hemolytic jaundice. The consensus is that determination of this factor is of no practical value in differentiating between hepatocellular and obstructive jaundice, and has little or no advantage over the qualitative van den Bergh reaction in the diagnosis of hemolytic jaundice. Nevertheless, in our experience, it is frequently very helpful in the study of patients with hepatic or biliary tract disease because abnormally high values for this component may occur with total serum bilirubin values within normal limits.

Whereas any level of one-minute, direct-reacting bilirubin above 0.24 mg./100 cc. is abnormal, a total serum bilirubin between the wide limits of 0.1 to 1.0 mg./100 cc. must be regarded as possibly normal. Inasmuch as about 75 per cent of normals fall below 0.8 mg. and 50 per cent below 0.5 mg./100 cc., hyperbilirubinemia is probably present in 75 per cent of cases at levels of 0.8 to 1.0 mg. and in 50 per cent of those between 0.5 and 1.0 mg./100 cc. It is in these ranges of bilirubinemia, particularly 0.8 to 1.0 mg./100 cc., that the one-minute direct measurements are of greatest clinical value. Regardless of the total bilirubin concentration, one-minute, direct-reacting bilirubin levels above 0.24 mg. are abnormal, reflecting impaired hepatic function or biliary stasis. Under such circumstances, the implication is that the total bilirubin level, although within normal limits, is abnormal for the subject in question, a situation analogous to BMR values within the range plus 10 to minus 10.

This determination is of little clinical value in the presence of serum bilirubin concentrations above 1.0 mg./100 cc., i.e., with frank hyperbilirubinemia, except in the diagnosis of hemolytic jaundice. It is indicated particularly in the study of patients with suspected hepatic disease (e.g., cirrhosis, hepatitis) with serum bilirubin concentrations within normal limits. It is useful also during convalescence from acute hepatic disease, e.g., infectious hepatitis, elevated values often persisting for some time after the total bilirubin has returned to normal levels, indicating residual liver functional impairment, and, perhaps, liver damage.

Extrahepatic Obstructive Jaundice. In experimental animals, when the pressure in the bile ducts is increased to a level higher than the secretory pressure of the liver, bilirubin usually appears in the thoracic duct lymph in ten to twenty minutes and increases in the blood of the systemic circulation usually after twenty minutes. It appears probable that, as a result of the obstruction, bilirubin diffuses from the bile capillaries to the hepatic lymph spaces and from the latter to the adjacent blood capillaries. A negative direct van den Bergh reaction is obtained during the first two hours and a positive direct reaction in two to four hours after ligation of the common duct. The initial negative direct reaction may be due to temporary reflex inhibition of hepatocellular function comparable to the reflex anuria which at times follows ligation of one ureter. It may also be due to functional saturation of the hepatic cells with bilirubin and a diminished capacity for removing the pigment from the blood. The subsequent diffusion of direct-reacting bilirubin from the bile canaliculi into the lymph spaces and blood capillaries results in the production of the direct reaction in the blood serum. The accumulation of indirect-reacting bilirubin in the blood due to inability of the liver cells to remove it adequately constitutes one form of "retention jaundice," while diffusion of direct-reacting bilirubin from the bile canaliculi into the lymph and blood has been termed "regurgitation jaundice."

Both direct- and indirect-reacting bilirubin increase in the blood of patients with moderate and severe jaundice due to extrahepatic biliary obstruction. If the view can be maintained that bilirubin in the blood which does not give the direct van den Bergh reaction has not passed through the hepatic cells, while that which does give the reaction has been reabsorbed from hepatic cells or from the bile canaliculi, it would appear that in the great majority of cases of obstruction the jaundice is a combination of the "regurgitation" and the "retention" varieties. The qualitative direct van den Bergh reaction may be negative in the first few hours of obstruction and, occasionally, following relief of the obstruction, after the serum bilirubin has fallen to about 1.5 mg. per 100 cc. The direct reaction may persist, however, for some time after the serum bilirubin has fallen to within normal limits in some cases.

Hepatocellular Jaundice. This type of jaundice, sometimes called nonobstructive hepatic jaundice, is due to hepatic cell damage usually resulting either from infection, directly or indirectly, or from chemical agents.

In certain of the infectious diseases, such as viral hepatitis (infectious hepatitis (IH); serum hepatitis (SH)), spirochetal jaundice (Weil's disease) and yellow fever, hepatic damage and jaundice are dominant features; in others (Table 38), hepatic involvement is an incident in the course of a generalized infection. In some instances, hepatocellular damage is accompanied and may be aggravated by excessive hemolysis, as in infections with hemolytic streptococci, staphylococci and pneumococci, malaria, Clostridium perfringens, and so on, and in favism.

Hepatotoxic chemical agents may act in one or more of three ways:

(1) Direct injury to liver cells: ether, chloroform, carbon tetrachloride, avertin, carbon disulfide, sulfonamides, dinitrophenol, nitrobenzene, trinitrotoluene, tetrachlorethane, synthalin, cinchophens, acriflavine, arsenic, phosphorus, manganese, tannic acid, amanitotoxin and bean poison (favism).

(2) Hemolysis, with injury to liver cells, either directly or by products of hemolysis (red cell stroma): phenylhydrazine, sulfonamides, trinitrophenol, arsine, toluenediamine, snake poisons, saponins, incompatible blood, hemolytic sera, bean poison.

(3) Hypersensitivity, idiosyncrasy, allergy, arsphenamines, cinchophen, mercury, bismuth, sulfonamides, liver extract, and a number of other agents employed for therapeutic purposes.

Hepatocellular damage, with jaundice, has been observed after x-ray overdosage and in eclampsia and severe burns (tannic acid therapy?). This type of hyperbilirubinemia may occur also occasionally in diabetes mellitus and rather commonly in congestive heart failure, erythroblastosis fetalis and cirrhosis of the liver. In erythroblastosis fetalis, and probably in congestive heart failure, it is contributed to by increased bilirubin formation resulting from excessive hemolysis.

The essential feature of hepatocellular jaundice is functional insufficiency of the hepatic cells. Under normal conditions bilirubin diffuses from the hepatic sinusoids into the perivascular spaces and is taken up by the hepatic cells and passed into the bile canaliculi. In the presence of hepatocellular damage the liver cells are unable to remove the required amount of bilirubin, which consequently accumulates in the blood. At this stage the qualitative direct van den Bergh reaction is negative, but the one-minute, direct-reacting bilirubin is often increased. As the condition progresses, hepatic cell damage increases (degeneration, necrosis, desquamation), the continuity of the bile canaliculi is disrupted and their lumen may be occluded by desquamated and disintegrated cells (bile thrombi). As a result, some bilirubin which has entered the canaliculi may pass back into the perivascular spaces and blood capillaries, with the production of a positive direct van den Bergh reaction in the blood serum. In all but very mild cases of hepatocellular jaundice, the reaction is therefore practically identical with that in obstructive jaundice, differing however, in this respect: at the onset of the disease, the negative direct reaction may persist for a longer time than in obstructive jaundice. In hyperbilirubinemia of this type, as exemplified by hepatitis, cirrhosis, congestive heart failure and sulfonamide poisoning, there is usually an increase in both direct and nondirect-reacting bilirubin, as in obstructive jaundice, indicating the operation of both retention and regurgitation mechanisms in its pathogenesis. An increase in one-minute, direct-reacting bilirubin may occur, e.g., in infectious hepatitis, before the total serum bilirubin rises above the upper limit of normal, and may be the only demonstrable abnormality in the serum bilirubin in mild cases and also for prolonged periods in patients with cirrhosis. Elevated values are also obtained frequently during convalescence from infectious hepatitis and other types of acute hepatic damage for some time after the total serum bilirubin concentration has fallen to normal levels. This should be interpreted as indicative of persisting liver functional impairment and, presumably, hepatic damage (p. 525).

A familial type of jaundice has been described with serum bilirubin concentrations as high as 13 mg. per 100 cc. and a negative direct van den Bergh reaction, in which the capacity of the hepatic cells for excreting bilirubin seems to be impaired ("constitutional hepatic dysfunction"). There is no evidence of excessive hemolysis or hepatic disease, nor of any impairment of liver function other than that of excreting bilirubin. A condition of "hereditary jaundice" of apparently identical nature has been described in rats.

Hemolytic Jaundice. This is observed clinically as a result of excessive hemolysis in a number of conditions, some of the most important of which are enumerated in Table 38. Hemoglobin injected intravenously or intraperitoneally is promptly converted to bilirubin. It is probable that normal hepatic cells are capable of handling efficiently much more bilirubin than they are called upon to excrete under normal circumstances. For the production of hyperbilirubinemia due to excessive hemolysis, it is probably essential that there be also some degree of impairment of hepatic cell function. This functional insufficiency is believed to be due usually to anoxemia, which is present constantly in such conditions. There is evidence that protein depletion also may impair the capacity of the liver for excreting abnormally large amounts of bilirubin.

In uncomplicated cases, and in the absence of severe morphologic damage to the liver cells, the serum gives a negative direct van den Bergh reaction. However, as indicated previously (p. 526) and in Table 37, severe anoxia, or the products of hemolysis, especially the erythrocyte stroma material, may cause serious hepatocellular damage with the production of a superimposed regurgitation type of jaundice and a positive direct van den Bergh reaction in the serum. In congenital hemolytic jaundice (spherocytic jaundice), the rather common complication of biliary calculi may be responsible for the same phenomenon.

Serum bilirubin values above 6 mg. per 100 cc. are unusual and above 10 mg. rare in uncomplicated hemolytic jaundice. It has been found that in icterus neonatorum and congenital hemolytic jaundice the increase in serum bilirubin is entirely in the nondirect-reacting form of pigment. In pernicious anemia, in which hyperbilirubinemia is probably not, strictly speaking, due to excessive hemolysis, there is a rather consistent increase in direct-reacting bilirubin, but not to the same extent as in the nondirect-reacting, although the qualitative direct reaction is invariably negative in this condition in the absence of complications.

Bilirubinuria

Nondirect-reacting bilirubin, i.e., that present in the blood plasma normally and in hemolytic jaundice, does not diffuse readily through normal capillary walls, presumably because it is bound rather firmly to a globulin, a molecule of rather large size (p. 520). Consequently, bilirubin does not appear in the urine (no bilirubinuria) in uncomplicated hemolytic jaundice, even with serum bilirubin concentrations as high as 10 to 15 mg./100 cc. In such cases there is no increase in direct-reacting bilirubin in the blood.

In hepatocellular and obstructive jaundice, the more diffusible, loosely-bound (p. 520), direct-reacting bilirubin enters the blood stream in abnormally large amounts, and is excreted in the urine. The view was formerly prevalent that there is a rather fixed "renal threshold" for bilirubin, analogous to that for glucose (p. 59), bilirubinuria occurring, in regurgitation types of jaundice, only when the serum bilirubin concentration rose to 1.6 to 2.0 mg./100 cc. This is now known not to be the case, at least not invariably, inasmuch as bilirubinuria may occur at normal serum bilirubin levels, e.g., in the early, pre-icteric stage of infectious hepatitis. The appearance of bilirubin in the urine correlates more closely with the level of direct-reacting than of total serum bilirubin, but this relationship is by no means consistent. For example, differences are observed at different stages of infectious hepatitis; in the early stage, bilirubinuria may occur with little or no significant increase in direct-reacting bilirubin ($<$ 0.4 mg./100 cc.), whereas later, during subsidence of the hyperbilirubinemia, it may disappear at direct-bilirubin levels in the serum as high as 1.2 mg./100 cc.

Because of its simplicity, a quantitative adaptation of the methylene blue test for bilirubinuria has been employed widely in the study of patients with hepatic and biliary tract disease. Positive tests, interpreted as indicating abnormal bilirubinuria, have been obtained in a large proportion (40 per cent) of patients with infectious hepatitis one to six days before the onset of clinical icterus and frequently in the presence of normal serum icterus index values. However, there is some danger in interpreting results with this procedure, since the evidence indicates that it is nonspecific, the positive green color obtained in urine containing bilirubin being due chiefly to a blend of yellow and blue colors. The development of a positive reaction is therefore influenced by concentration or dilution of the urine and by any yellow constituent. A positive test is obtained with artificially produced bilirubinuria at concentrations of 2 mg. or more of bilirubin per 100 cc. of urine. Despite its nonspecificity, the simplicity of the procedure makes it useful in large scale testing during epidemics of infectious hepatitis and where complete laboratory facilities are lacking.

More specific, sensitive tests for bilirubinuria (e.g., Harrison spot test) have been employed to advantage under similar circumstances with somewhat better results than the methylene blue test. This procedure has been found to yield a positive result (bilirubinuria) in a considerable proportion of cases of infectious hepatitis before a significant increase was observed in either the total or the prompt-direct-reacting serum bilirubin concentration, as indicated above.

Bilins and Bilinogens in the Urine and Feces

The relationship between the various derivatives of hemoglobin is illustrated in Figure 52, and has been described previously (p. 516). The pertinent facts regarding the metabolism of urobilinogen may be summarized briefly as follows.

Under normal conditions bilirubin entering the intestine in the bile is transformed, through the action of bacteria, into mesobilirubinogen and stercobilinogen (collectively termed bilinogens, or urobilinogen), colorless substances, which are subsequently oxidized to urobilin and stercobilin. The former ("bilinogens") will be collectively referred to as urobilinogen, and the latter ("bilins") as urobilin. Both are present in the feces and, in traces, in the urine of normal subjects. They are usually not present in fresh bile of normal individuals in more than minimal amounts, but may appear in the bile in patients with hepatic disease. A portion of the urobilinogen formed in the intestine is reabsorbed into the portal blood stream and carried to the liver where it is metabolized, the products being unknown. This cycle is termed the enterohepatic circulation of bile pigment. The following conclusions seem warranted on the basis of available evidence:

(1) Bacterial infection or putrefaction is necessary for the conversion of bilirubin to urobilin. This may occur in the intestine (normal) or, theoretically, at least, in the biliary passages in the presence of infection.

(2) In conditions of moderate hepatic damage, incomplete biliary obstruction, cholangitis and hemolytic jaundice the liver is unable to metabolize completely all of the bilirubin absorbed from the intestine and some consequently passes into the general circulation and is eliminated in the urine. Under such circumstances urobilinuria is an indication of defective hepatic function. In pure hemolytic icterus, although the function of the liver may be unimpaired, it cannot metabolize completely the excessive amounts of bilirubin formed from the increased quantity of bilirubin which passes into the bile.

Urobilinogen in Feces. The term "urobilinogen" is applied here, and in subsequent sections, to the total bilins and bilinogens, combined, for purposes of convenience. This is permissible because these substances have the same clinical significance. The accurate quantitative estimation of the urobilinogen content of the feces is not employed routinely clinically but may be of great value in certain conditions. The normal daily excretion in the feces of adults ranges from 40 to 280 mg. (usually 100–200 mg.). The urobilinogen content of the feces or duodenal contents appears to be an index of the degree of blood destruction in the absence of hepatic or biliary tract disease. Increased quantities have been found in the duodenal bile and feces in hemolytic jaundice, paroxysmal hemoglobinuria and other hemolytic processes (Table 38) and during periods of relapse in pernicious anemia. In the latter condition, during remissions induced by liver therapy, the urobilin content of the feces drops to within normal limits. The urobilinogen content of the feces of children is low as compared to that of adults, averaging less than 3 mg. daily up to the age of eleven years.

Low values may be obtained in the presence of inanition, inactivity, low-grade infections, hypochromic anemia and certain cases of secondary hyperchromic anemia. Inasmuch as the quantity of urobilinogen in the feces depends primarily upon the quantity of bilirubin entering the intestine, variations in the former in hepatic and biliary tract disease are determined largely by (1) the degree of impairment of bilirubin excretion by the hepatic cells, (2) the presence and degree of obstruction to the flow of bile and (3) the presence and severity of an associated hemolytic process.

This determination may be of value in distinguishing between jaundice due to stone in the common duct and that due to neoplasm of the duct, duodenum or head of the pancreas. Theoretically, in the absence of marked biliary tract infection, complete obstruction of the common duct, with consequent absence of bile pigment from the intestine, should result in a total absence of urobilinogen from the feces and urine. This fact is applied particularly to the differentiation between common duct obstruction due to stone, which is seldom permanently complete, and that due to neoplasm, which usually becomes permanently complete. Absence of urobilinogen from the urine, a quite constant finding in obstructive jaundice due to neoplasm, may occur also at times with obstruction due to stone. However, excretion of urobilinogen in the feces usually differs strikingly in these two groups of patients, being frequently within normal limits (10–250 mg. daily) in the patients with calculous obstruction, and usually low or extremely low or absent (0–5 mg. daily) in those with malignant obstruction.

In the presence of severe, diffuse liver damage, e.g., in infectious hepatitis and acute necrosis, there may be periods, usually relatively brief, in which no bile enters the intestine (acholia). In this condition of total suppression of bile formation, bilins and bilinogens will be absent from the feces (and also urine), as in complete biliary obstruction.

Urobilinogen in Urine. As stated above, absence of urobilinogen from the urine in patients with jaundice is indicative of complete obstruction of the common duct or of complete suppression of bile pigment excretion by the liver. This finding may also be present, whether or not jaundice is present, in patients with bile fistula in whom the bile is draining either externally or into some viscus other than the intestine. However, some urobilinogen may be present in the urine occasionally in cases of complete obstruction of the common duct. This may occur particularly in cases of calculous obstruction with marked infection of the bile passages or obstructive biliary cirrhosis and, rarely, in patients with marked jaundice in whom some bile pigment may pass into the lumen of the bowel directly from the jaundiced intestinal mucous membrane.

One to 4 mg. of urobilinogen are eliminated daily in the urine by the normal adult. According to the less accurate methods commonly employed (Wallace and Diamond), a positive reaction for urobilinogen may be obtained normally with urine dilutions up to 1 to 20; a positive reaction with dilutions above this figure is considered indicative of the presence of excessive quantities. Slight increases may occur in subjects without hepatic, biliary tract, or hemolytic disease in the presence of constipation, allowing an abnormally prolonged period of reabsorption of urobilinogen from the intestine. Although in pathologic conditions the morning urine may contain relatively large quantities of urobilinogen, the rate of elimination varies so much from hour to hour and day to day that all estimations should preferably be made on twelve or twenty-four-hour specimens and should be repeated for at least five consecutive days before a negative reaction is interpreted as indicating the absence of urobilinuria.

Excessive amounts of urobilinogen and urobilin are found in the urine under the following circumstances:

Excessive Hemolysis. Excessive urobilinogenuria occurs characteristically in congenital and acquired hemolytic jaundice, pernicious anemia, splenic anemia and other conditions associated with hemolytic features (Table 38), due to the fact that the liver is unable to deal with the excessive amount of urobilinogen formed from the abnormally large quantity of bilirubin in the intestine. A sudden increase in urine urobilinogen has been observed shortly after the occurrence of pulmonary infarction in patients with congestive heart failure or auricular fibrillation, due presumably to hemolysis in the infarcted areas. It has been found that patients with pernicious anemia in whom excessive urobilinuria disappears before the reticulocyte crisis (induced by treatment) exhibit a greater increase of hemoglobin and red blood cells than those in whom excessive urobilinuria persists for even a short time after the reticulocyte crisis. Some believe that excessive urobilinuria in these anemic conditions is due in part to some disturbance of hepatic function, attendant perhaps upon the existing state of anoxemia. Similar findings have been obtained during malarial chills. It is believed by some that the total urobilinogen excretion may be regarded as a quantitative index of the amount of hemoglobin destruction (p. 516).

Hepatic Functional Impairment. (*a*) *Hepatocellular Jaundice.* Urobilinogenuria occurs regularly in the presence of hepatic functional impairment as long as adequate quantities of bilirubin are entering the bowel. This phenomenon occurs, therefore, in the great majority of patients with jaundice due to parenchymal hepatic lesions, including hepatitis, acute and subacute hepatic necrosis, toxemia of pregnancy, arsphenamine, chloroform and carbon tetrachloride poisoning, congestive heart failure and liver damage due to a great variety of other toxic and infectious agents. In animals poisoned with chloroform, it has been found that if the hepatic damage is so severe that no bilirubin is excreted by the liver, urobilinogenuria does not occur. This finding coincides with the common clinical observation of excessive urobilinogenuria in the very early and late stages of acute hepatitis, including so-called infectious hepatitis, while it is absent during the stage of complete suppression of bilirubin excretion or obstruction to its excretion due to cholangitis. We have observed excessive urobilinogenuria in the early stages of acute and subacute hepatic necrosis which disappeared as the condition progressed and the degree of bilirubinemia increased with the development of such marked hepatocellular damage that no bile pigment was eliminated.

Excessive urobilinogenuria is a sensitive index of the presence of liver dysfunction. It frequently persists after all other evidence of hepatic damage has disappeared and may appear before the development of any other manifestation of such damage.

(*b*) *Hepatic Disease Without Jaundice.* Excessive urobilinogenuria occurs commonly in portal cirrhosis even in the early stages of its development. In this condition, it is due in part to hepatic functional impairment and, in addition, to the fact that a portion of the portal blood reaches the general circulation without having passed through the liver. In this condition, excessive urobilinogenuria may occur in the absence of hyperbilirubinemia. Urobilinogenuria has been frequently observed in patients with congestive heart failure, considerable prognostic significance being attributed to it by some authors. In this condition, it is probably dependent upon the presence of hepatic damage, functional or organic, resulting perhaps partly from a state of prolonged anoxemia incident to the existing circulatory disturbance, and partly from injury to the polygonal cells by pressure from the dammed-back blood. It usually precedes the development of hyperbilirubinemia. It may also be observed in the absence of jaundice in certain toxic states, particularly pneumonia, streptococcus infections and malaria, due partly to excessive hemolysis and partly to hepatic parenchymal damage. Because of the nature of the conditions responsible for its occurrence, excessive urobilinogenuria is regarded by some as of great prognostic significance in pneumonia. This finding has also been obtained in patients with cholecystitis and cholelithiasis in the absence of hyperbilirubinemia, due to an associated mild hepatitis.

(*c*) *Obstructive Jaundice.* It now appears that most of the discordant experimental observations regarding the question of the formation of urobilinogen in the absence of bilirubin from the intestine may be explained on the basis of the presence or absence of active infection of the bile passages in the experimental animals. Except in the presence of such infection, urobilinogenuria rarely if ever occurs in complete obstructive jaundice. As was mentioned, in some cases of profound jaundice, a small quantity of urobilinogen may be formed from bile pigment which has passed from the jaundiced intestinal mucosa into the lumen of the bowel. It has been shown experimentally that bilinogens are not present in the urine in complete biliary obstruction even though the liver be simultaneously severely damaged by chloroform, phosphorus or other hepatotoxic agents. It has frequently been observed that urobilinogenuria, occurring in patients with cholelithiasis, disappears completely with the development of complete obstruction of the common bile duct. When the bile flow is reestablished, urobilinogenuria reappears. This phenomenon is observed not only in cholelithiasis but also in hepatitis with associated cholangitis, e.g., in infectious (viral) hepatitis. Urobilinogenuria may be observed in infective cholangitis, especially when a gallstone is floating in the common duct. It may also be encountered occasionally in patients with complete calculous obstruction complicated by purulent cholangitis. Such cases are, however, not common, and it may be assumed generally that in the majority of cases of obstructive jaundice the presence of urobilinogen in the urine suggests that the obstruction is not permanently complete. Under such circumstances, excessive urobilinogenuria is frequently encountered, dependent upon associated marked hepatic functional impairment with consequent inability of the liver to remove from the blood even relatively small quantities of urobilinogen absorbed from the bowel.

Urobilinogenuria is not pathognomonic of any single morbid condition, its chief diagnostic significance, apart from its occurrence in hemolytic anemia, pernicious anemia and chronic hemolytic jaundice, being that some bile pigment is entering the intestine and that the functional activity of the liver is either very considerably impaired or that there is active infection in the bile ducts. When properly interpreted, the study of urobilinogen excretion is of great value not only in the study of the state of hepatic function but also in differentiating between complete and incomplete obstruction to the flow of bile. This may be of distinct value in distinguishing between jaundice due to stone in the common duct and that due to neoplasm of the duct, duodenum or head of the pancreas. In the latter connection, simultaneous investigation of the urobilinogen excretion in the feces is of great value (p. 530). Occasionally, complications such as severe, protracted diarrhea (decreased absorption of bilinogens from intestine) or renal insufficiency (decreased renal elimination of bilinogens) may interfere with the usual interpretations of changes in urine urobilinogen.

Bile Pigments in Feces

Inasmuch as bilirubin is practically completely converted to "bilinogens" and "bilins" in the intestine, the latter constitute the normal pigment of the feces, bilirubin being rarely present, except occasionally in severe diarrhea with extremely rapid passage of material through the intestine, and in fistulous communications between the upper and lower bowel. Bilirubin is also present in the feces of normal infants during the first 8 to 75 days of life. The persistent absence of pigment from the feces is indicative of obstruction to the outflow of bile from the liver or of complete suppression of bile pigment excretion by the liver. Complete obstruction of the bile passages may occur in cholangitis but is most commonly associated with stone in the common duct, stricture of the bile ducts, carcinoma of the head of the pancreas, common duct or duodenum or, rarely, enlargement of the lymph nodes in the region of the common bile duct. The feces are pale, having a so-called "clay-colored" or putty-like appearance, and are usually bulky, extremely offensive and contain excessive amounts of neutral fat and fatty acid (p. 513). If the obstruction is not complete the feces may be fairly normal in color, and even in complete obstruction some urobilin may be present in the feces due to the entrance of small amounts of bile pigment into the intestine from the capillaries of the intestinal mucous membrane.

The significance of accurate quantitative estimations of the "urobilinogen" content of the feces in this connection has been referred to elsewhere (p. 530). It must be remembered, too, that so-called "clay-colored" feces may actually contain considerable amounts of pigment, the presence of which is obscured by large amounts of fat. Before deciding that pigment is absent from the feces under such circumstances, the fat should be removed by extraction. Of greater value in determining whether or not bile is entering the intestine is the procedure of duodenal intubation, the tube being allowed to remain in situ for periods of several hours on successive days.

Porphyrin in Urine and Feces

The pertinent facts regarding normal porphyrin excretion have been outlined elsewhere (p. 204). Under normal conditions the daily excretion of coproporphyrin is about 60 to 280 (av. 160) micrograms in the urine and 300 to 1100 micrograms in the feces, the normal urine : feces ratio being about 1 : 5. About 60 to 80 per cent of the urinary coproporphyrin and 70 to 90 per cent of the fecal coproporphyrin is in the form of the Type I isomer, the remainder being Type III.

Increased porphyrinuria has been reported in patients with cirrhosis, hemochromatosis, hepatitis, melanosarcoma of the liver, acute and subacute hepatic necrosis, chronic passive congestion of the liver, obstructive jaundice, infectious hepatitis and metastatic carcinoma of the liver. This is due largely to inability of the liver to excrete the porphyrin in the bile. In the absence of biliary obstruction, an increase in the urine : feces porphyrin ratio may be of more significance in indicating impairment of hepatic function than the absolute amount excreted in the urine. This ratio has been found to be 0.8 to 22.0 in patients with hepatic disease. In these conditions, except those associated with alcoholism and heavy metal (lead, arsenic) poisoning, the increase in urine porphyrin is largely in Type I coproporphyrin, i.e., that which predominates normally (Table 6, p. 211). Interestingly, in acute alcoholism, and in cases of cirrhosis occurring in alcoholics, the increase is mainly in Type III coproporphyrin, in contrast to the Type I porphyrinuria which occurs in infectious hepatitis and in cirrhosis in nonalcoholic subjects.

Determination of urine coproporphyrins is of no value in differentiating hepatocellular from extrahepatic obstructive jaundice, being rather consistently increased in both. This increase may persist after apparent clinical recovery and after the results of other tests of liver function have returned to normal. Moreover, as indicated above, determination of the type of porphyrin involved may be useful in distinguishing "nonalcoholic" cirrhosis (e.g., following infectious hepatitis) from "alcoholic" cirrhosis, and in detecting hepatic disease occurring as a result of heavy metal poisoning.

Apart from these acquired diseases of the liver and biliary tract, there is a form of porphyrinuria, termed "porphyria hepatica," which comprises the largest group of the so-called congenital porphyrias (p. 210). This shows a familial incidence, and is characterized by a variety of neurological, psychiatric, abdominal, and cutaneous (photosensitivity) manifestations, associated with evidence of impaired liver function, including jaundice, at times. The relation of the liver disturbance to the porphyria is not established. Some believe that intercurrent liver dysfunction, (e.g., in fatty liver or cirrhosis), alcoholism, or barbiturate intoxication, is responsible in such cases for "activation" of an inherited condition of latent porphyria. In this condition, the urine contains a marked excess of coproporphyrin, mainly Type III, an excess of uroporphyrin of both types, and a nonfluorescing compound, "porphobilinogen," which may be a precursor of the porphyrins. The liver also contains excessive amounts of porphyrins.

Other types of porphyrinuria are reviewed elsewhere (p. 210). These must be considered in interpreting alterations in urine coproporphyrin in terms of liver function or hepatic disease.

BILE ACID METABOLISM

The bile acids, glycocholic and taurocholic acids, are present in the bile and blood as their sodium salts, the relative proportions of the two substances varying in different individuals. Isotope studies have shown that cholic acid can be formed in the organism from cholesterol, and the latter from acetate, indicating an intimate metabolic relationship between bile acids, cholesterol and fatty acids. In the liver, cholic acid is conjugated with glycine and taurine (from cystine) to form glycocholic and taurocholic acids. The chemical relation of bile acids to sex hormones, vitamin D, adrenal cortical hormones, cardiac glucosides, and certain carcinogenic hydrocarbons is referred to elsewhere (p. 84).

The bile acids are formed in the liver. When isotopically labeled cholesterol is injected intravenously, about 60 to 90 per cent is apparently converted to bile acids in the liver and excreted in the bile. This transformation therefore appears to be one of the major pathways of metabolism of the cholesterol of the plasma, which itself originates in the liver (p. 99).

The quantity of bile acids formed and excreted varies widely, depending presumably upon the intensity of stimulation of this function by the digestive mixture in the intestine, the capacity of the liver in this regard being very large. The total daily excretion has been found to vary from 5 to 10 grams. In the intestine, the bile salts are absorbed into the portal circulation, from which they are largely removed by the liver and re-excreted in the bile (enterohepatic circulation). A small amount escapes removal by the liver, passing into the systemic circulation, in which cholate is present in concentrations of 0.2 to 3.0 mg./100 cc.

The most important functions attributed to the bile salts are:

1. They facilitate digestion of fats by their emulsifying action (lowering sur-

face tension), thus increasing enormously the surface area of the fat exposed to the action of pancreatic lipase.

2. They aid in absorption of fatty acids, cholesterol, carotene (provitamin A), and the fat-soluble vitamins D and K, presumably by forming complexes more soluble in water ("hydrotropic" action).

3. They are the most potent natural stimulus to hepatic bile flow, increasing several-fold the volume flow of bile (choleretic action), the output of solids being, however, not affected.

4. They aid in keeping cholesterol in solution in the bile. When the bile acid: cholesterol ratio falls below a critical level, cholesterol may precipitate. This may be a factor in the pathogenesis of biliary calculi.

Because of technical difficulties, the quantitative estimation of bile salts in the blood is not commonly employed in the study of patients with hepatic or biliary tract disease. It has been found, however, in clinical and experimental obstructive jaundice, that bile salts appear in the blood and increase for some time during the early period of stasis. After prolonged obstruction, the concentration of bile salts in the blood diminishes, due presumably to diminished synthesis of these substances as a result of progressive hepatocellular damage. A similar secondary decrease in the bile salt concentration of the blood in animals with complete biliary obstruction has been observed following the administration of hepatotoxic substances, such as chloroform, carbon tetrachloride and tetrachlorethane. Although there have been occasional reports of an increase in bile salts in the blood of patients with portal cirrhosis, carcinoma of the liver and other hepatic diseases, these findings have not been substantiated by recent clinical and experimental studies. The concentration of these substances in the blood appears to increase only in the early stages of biliary stasis and not in the presence of hepatocellular damage in the absence of obstruction to the flow of bile through the extrahepatic bile passages. Intravenously injected bile salt (sodium cholate) remains in the systemic circulation longer in patients with obstructive or hepatocellular jaundice than in normal subjects. The rate of removal is slower in hepatocellular than in obstructive jaundice. This has been proposed as a means of distinguishing between these conditions.

From a practical standpoint, alterations in bile salt metabolism are investigated chiefly, if at all, by determining the presence or absence of these substances in the urine and their concentration in the bile, the latter particularly in patients with bile fistula. In obstructive jaundice, the increase in the bile salt concentration of the blood is accompanied by the appearance of the salts in the urine. The development, in such cases, of marked hepatocellular damage, whether due to prolonged stasis, superimposed hepatitis or the administration of hepatotoxic agents, is followed by a fall in the bile salt concentration of the blood and, in some cases, a disappearance of bile salts from the urine. The presence of bile pigment in the urine in the absence of bile salts is termed dissociated jaundice. The same phenomenon, constituting another type of dissociated jaundice, occurs occasionally in patients with hemolytic icterus.

The importance of certain abnormalities in the composition of bile is discussed elsewhere (p. 556). From a practical standpoint, investigation of the bile salt content of bile may be of value in patients with bile fistula or with a drainage tube in the common duct following operation upon the bile passages. It has been found that bile salts are invariably absent from the liver bile in cases in which the common bile duct has been obstructed for a week or more. A period of one to four weeks or longer may elapse between the time of release of obstruction and the reappearance of bile salts in the liver bile. This interval appears to be roughly proportional to the degree and duration of obstruction and the degree of cholangitis associated with the obstruction. The persistent absence or very low concentrations of bile salts in the drainage bile under such circumstances is of poor prognostic significance, indicating severe hepatocellular damage. The serious significance of this phenomenon is indicated by the fact that the liver is able to synthesize bile acids in essentially normal amounts even in the face of extensive hepatic damage, as in advanced portal cirrhosis. There is some evidence that the failure to demonstrate bile salts in the bile under such circumstances is due to the presence of abnormal forms, perhaps representing intermediate stages in the synthesis of cholic acid, which do not respond to the Pettenkofer reaction.

DETOXIFICATION-CONJUGATION

The liver plays an important part in protecting the organism from various toxic substances entering through the intestinal tract. This "detoxifying" function is believed to involve processes of oxidation and conjugation into relatively nontoxic substances which are subsequently eliminated in the bile and urine. For example, indole absorbed from the intestine reaches the liver in the portal circulation, is oxidized to indoxyl, conjugated with sulfuric acid and eliminated in the urine as indoxyl sulfuric acid. Other substances, including salicylic acid, menthol, camphor, phenol, etc., are in this way transformed into conjugate glycuronates and excreted in the urine. The estimation of inorganic and ethereal sulfates in the blood and urine and of conjugate glycuronates or sulfates in the urine following the administration of such substances has been proposed as a method of estimating liver function. These tests have proved to be of little clinical value. The term "detoxification" is in a certain sense a misnomer. It has come to be applied, without regard to the toxicity of the compound involved, to those chemical changes occurring in the body which convert exogenous or endogenous compounds of known chemical structure to derivatives which are excreted in the urine. These include certain hormones, such as the female sex hormones, progesterone and estrone.

In recent years, investigation of the ability of the organism to synthesize hippuric acid and to oxidize cinchophen has been proposed for the study of the detoxifying and conjugating activity of the liver. The hippuric acid excretion test has been widely employed for this purpose.

Hippuric Acid Synthesis. This test is based upon the ability of the organism to conjugate glycine and benzoic acid to form hippuric acid, which is eliminated in the urine. The site of synthesis of hippuric acid has been the subject of considerable controversy; early experimental observations suggested that it has its origin in the kidneys. In man, the liver is perhaps the principal site of formation of this substance. The hourly rate of excretion of hippuric acid in the urine of normal subjects following the ingestion of benzoic acid is remarkably constant, being influenced somewhat by the body surface area. In certain types of hepatic disease the output of hippuric acid is markedly reduced. This reduction is assumed to be due in part to a diminished capacity of the liver to synthesize glycine, which is essential for the formation of hippuric acid, and in part to damage of the enzymatic mechanism which unites benzoic acid with glycine. In man, this conjugating enzyme appears to be present chiefly in the liver, and the small amount which is present in the kidneys is regarded as insufficient to compensate for the hepatic defect in liver disease. The urinary elimination of hippuric acid after the ingestion of benzoic acid is therefore regarded as a measure of the capacity of the liver to furnish glycine (aminoacetic acid) and also as an index of its detoxifying or conjugating power.

A subject with a normally functioning liver excretes approximately 3 gm. of benzoic acid, in the form of hippuric acid (4.41 gm.), in the urine in four hours after the ingestion of 6 gm. of sodium benzoate. The normal range of variation has been established at 85 to 110 per cent of this figure. In many cases the intravenous route of administration of the sodium benzoate is preferable in order to obviate the possibility of error due to abnormalities of absorption from the intestine. Normal subjects excrete 0.7 to 0.95 gm. of benzoic acid (as 1.0–1.4 gm. hippuric acid) in the urine during the first hour after intravenous injection of 1.77 gm. of sodium benzoate. It has been found that the excretion of hippuric acid under these circumstances is influenced by the size of the subjects and the following equation has been suggested for calculating the predicted normal excretion:

Hippuric Acid (grams) $= 0.34 + (0.00668 \times \text{weight in pounds})$. Subnormal values have been obtained in patients with a variety of hepatic disorders, including infectious hepatitis, various other forms of hepatitis, hepatic syphilis, portal cirrhosis, biliary cirrhosis, metastatic carcinoma of the liver, and acute and subacute hepatic necrosis. It has been suggested that hyperexcretion of hippuric acid (120–165% of normal) may occur in mild forms of hepatic disease, as in hepatitis accompanying cholecystitis. Normal values have been obtained in uncomplicated obstruction of the common duct, abnormal findings being observed in cases of extremely long duration and in those with superimposed hepatocellular damage. The test may, therefore, be of value in differentiating between hepatocellular and obstructive types of jaundice.

Several authors have confirmed the value of this procedure in the study of hepatic function; however, it is necessary to investigate the state of renal function before attaching significance to abnormal findings in patients with hepatic disease. This is due to the fact that diminished or less rapid elimination of hippuric acid may be dependent upon impairment of kidney function, which is present frequently in such cases. It has been found also that synthesis and excretion of hippuric acid are subnormal in conditions other than nephritis and hepatic disease, among these being cachectic states, anemia and chronic passive congestion.

If the presence of these disturbing factors can be excluded, this test is, in our experience, one of the most useful of the available methods of studying hepatocellular function.

THE LIVER AND HORMONE METABOLISM

In view of the fact that liver cells contain enzyme systems capable of catalyzing a large number and wide variety of chemical reactions, it is not surprising that certain hormones undergo metabolic transformations in this organ. Demonstration of abnormalities in this area of hepatic physiology is of little or no practical value in the clinical study of liver function or in differential diagnosis. However, it seems desirable to review briefly certain phases of this subject that appear to have clinical implications.

In general, the action of the liver serves to limit the amount and activity of various hormones in the systemic circulation. This is accomplished by one or more of the following mechanisms: (*a*) transformation to hormonally inactive or less active substances ("inactivation"); (*b*) conjugation of these metabolites, usually with sulfate or glucuronate, which renders them more soluble in water and hence more readily excreted in the urine; (*c*) excretion of the hormone or metabolites in the bile, in some cases with subsequent partial reabsorption from the intestine (enterohepatic circulation).

The liver contains enzymes which inactivate insulin, thyroxine, and certain anterior and posterior pituitary hormones, the nature of the metabolites being unknown. There is evidence, too, that it may be involved in the metabolism of the adrenocortical hormones, e.g., in their transformation to 17-ketosteroids and to other inactive metabolites of unknown nature. It is believed that the liver is the organ chiefly involved in the conversion of progesterone to pregnanediol, a biologically inactive substance, which is in part perhaps excreted in the bile, in part degraded to unknown metabolites, and in part conjugated (glucuronate), passed into the blood stream and excreted in the urine (p. 409). However, the largest amount of clinically pertinent information in this field is concerned with the role of the liver in the metabolism of estrogens and androgens.

Testosterone, probably the major androgen produced in the testis, is believed to be metabolized in the liver (in part perhaps also in the kidneys) to unidentified compounds and to steroids of lower androgenic activity, the most important of which is androsterone, a 17-ketosteroid (p. 416). This substance is in part, at least in some species, excreted in the bile; the largest fraction, however, undergoes conjugation with sulfate, is passed into the blood stream, and is excreted in the urine in this form, together with a relatively small amount of free androsterone. About one-third of the 8 to 20 mg. of neutral 17-ketosteroids excreted daily in the urine of normal men is derived from the testis, the remainder originating in adrenocortical hormones. The latter are the only source of urinary 17-ketosteroids in women (5–15 mg. daily) (p. 416 ff.).

Changes in androgen metabolism have been demonstrated in subjects with hepatic disease, e.g., infectious hepatitis, chronic hepatitis, and cirrhosis. These include: (*a*) decrease in urinary 17-ketosteroids; (*b*) increase in free 17-ketosteroids in the urine, a reflection of inadequate conjugation; (*c*) subnormal concentration of 17-ketosteroids in the blood after intravenous injection of testosterone (inadequate metabolism). The rather consistent occurrence in such cases of an increase in urine estrogen and a decrease in gonadotrophin suggests that the lowered 17-ketosteroid output, which is often accompanied by testicular atrophy, may be secondary to an increase in circulating estrogen (depressing gonadotrophin secretion by pituitary). That inadequate metabolic transformation may play a role, however, is suggested by the observation that subjects with hepatic damage excrete a somewhat smaller proportion of exogenous testosterone as 17-ketosteroids than do normal subjects. The main defects in androgen metabolism in patients with severe liver damage therefore appear to be: (*a*) diminished secretion of testosterone by the testes, a consequence of decreased gonadotrophin secretion due to an excess of circulating free estrogen; (*b*) perhaps somewhat diminished transformation of testosterone to 17-ketosteroids (mainly androsterone); (*c*) diminished conjugation of androsterone (and other 17-ketosteroids).

The liver plays an important role in the metabolism of estrogens. It contains enzyme systems which can convert estradiol, the primary and most active estrogen, in part to biologically inactive substances and in part to estrogens of lower activity, i.e., estrone and perhaps estriol. Conjugation of the estrogens is also believed to take place in the liver, with the formation and transfer to the blood stream of water-soluble glucuronidates and sulfates, which are ultimately excreted in the urine. Relatively large amounts of free estrogen are present in the bile in certain species, suggesting that biliary excretion may play an important role in the removal of estrogens from the blood stream. Estrogens may subsequently undergo an enterohepatic circulation, being gradually degraded in the liver to compounds of lower activity. There is evidence, too, that estrone may be converted in this organ to the more potent estradiol, a process of biological "activation."

The presumed functions of the liver in this connection may be summarized as follows: (1) it effectively removes free estrogens from the systemic circulation by (*a*) biliary excretion and (*b*) metabolic degradation to less active and inactive compounds; (2) it conjugates them with glucuronate and sulfate, converting them to water-soluble forms (inactive until hydrolyzed) which pass into the systemic circulation and are excreted in the urine; (3) it may possibly convert estrone to the more potent estradiol. The urinary estrogens, mainly glucuronidates and sulfates of estrone, estriol, and estradiol, are estimated as representing about 5 to 10 per cent of the activity of the estrogens originally secreted.

In subjects with hepatic disease, diminished activity of the liver in removing free estrogens from the blood stream, and in "destroying" and conjugating them, is reflected in the following phenomena: (1) increase in total urine estrogen; (2) increase in free estrogen in the urine; (3) delayed removal of exogenous estrogen from the blood and abnormally high urinary estrogen excretion following injection (decreased estrogen tolerance). Although an estrogen tolerance test has been proposed as a means of studying liver function, there is no close correlation between demonstrable abnormality of estrogen metabolism and the degree of impairment of liver function as measured by more conventional procedures. Moreover, we have found changes in subjects with extrahepatic duct obstruction similar to those obtained in the presence of hepatocellular damage, emphasizing the importance of impaired biliary excretion of estrogens in the production of these phenomena.

The abnormally high concentration of free estrogen in the blood of patients with hepatic disease is regarded as responsible for certain clinical manifestations that occur rather frequently in such cases, particularly in cirrhosis. These include: in women, menstrual disturbances (menorrhagia, metrorrhagia, amenorrhea), infertility, breast changes, and "spider" telangiectasis ("vascular spiders"); in men, testicular atrophy, decreased libido, impotence, infertility, axillary alopecia, vascular spiders, and gynecomastia.

ELIMINATION OF DYES

It has been found that certain dyes are removed from circulation and eliminated almost entirely by the liver, just as phenolsulfonephthalein is excreted almost entirely by the kidneys. Among these dyes are azorubin S., rose bengal, phenoltetrachlorphthalein, phenoltetraiodophthalein and sodium phenoltetrabromphthalein sulfonate (bromsulfalein). Several liver function tests have been proposed which have for their basis the estimation of the ability of the liver to eliminate these substances. These tests, as originally described, consisted in the determination of the quantity of dye recovered from the duodenal contents and feces following its injection into the blood stream. This method has now been largely abandoned, the procedure in common use at the present time consisting in the determination of the rate of removal of the dye from the blood stream, the disappearance rate being assumed to be an indication of the state of hepatic function.

Bromsulfalein (BSP) Excretion. Bromsulfalein is the dye most widely employed for this purpose. Its applicability to the study of liver function is based upon the assumption that its removal from the circulation is accomplished virtually exclusively by the liver. Studies in hepatectomized animals indicate that organs other than the liver can remove BSP from the blood stream, but, in the presence of a normal liver, probably not more than 5 per cent is removed by extrahepatic tissues. If there is marked retention of the dye in the plasma, as much as 10 per cent may be excreted in the urine, and considerably more in the presence of albuminuria (dye bound to plasma albumin). It has been claimed also that another source of error in interpreting results obtained with this procedure arises out of the fact that some of the BSP excreted in the bile is reabsorbed into the portal circulation, a small amount passing back into the systemic circulation. The concept of significant participation of the reticuloendothelial system in the primary removal of this dye from the blood stream has not received strong support. There seems to be little doubt that, under clinical conditions, the liver is the organ principally involved in this process, the relatively minor factors of extrahepatic removal, enterohepatic circulation, and renal excretion not interfering significantly with the interpretation of results of the BSP test in terms of liver functional efficiency.

Following a single intravenous injection of BSP, about 10 to 15 per cent of the amount in the blood is removed each minute (almost exclusively by the liver cells) and is excreted in the bile by a process of active secretion. The excretory mechanism closely resembles that for bilirubin. Excretion in the bile continues for some time after all dye has been removed from the blood stream in normal subjects. This suggests a biphasic mechanism, i.e., (*a*) primary removal from the plasma with temporary storage in liver cells, and (*b*) subsequent active secretion into the bile canaliculi. In certain cases, therefore, study of the curve of excretion of BSP in the bile (duodenal intubation) may yield information not given by the usual studies of the rate of its removal from the blood.

Employing the technic of hepatic vein catheterization in subjects receiving a continuous intravenous infusion of BSP, the hepatic blood flow may be estimated from determinations of: (1) the concentration of BSP in the hepatic vein blood and (2) the peripheral blood; (3) the amount of BSP removed by the liver. By this procedure, the hepatic blood flow in normal subjects has been found to be 1085–1845 cc./minute/1.73 sq. M body surface area, averaging 1500 cc., or approximately 100 cc./100 gm. liver. Inasmuch as the removal of BSP from the blood stream is dependent upon the volume flow of blood through the liver, it follows that abnormal retention of the dye may result from decreased hepatic blood flow as well as from impaired liver cell function.

Blood BSP Retention Test. As usually performed, this procedure consists in the intravenous injection of BSP in the dosage of either 2 mg. or 5 mg./per kg. body weight. Blood samples are withdrawn at desired times subsequently, the serum or plasma separated and alkalinized (to develop purple color), and the concentration of dye measured colorimetrically. Normal values at different time intervals are as follows:

Dose	5 min.	15 min.	30 min.	45 min.
2 mg./kg.	< 60%	< 10%	0	0
5 mg./kg.	< 85%	< 30%	< 10%	< 6%

The 2 mg. dosage possesses the advantage that any retention at thirty minutes is abnormal. The advantage of the 5 mg. dosage lies in the fact that it imposes a greater functional burden on the liver cells and, consequently, may yield abnormal results in cases of relatively mild functional impairment in which normal results may be obtained with the 2 mg. dosage. Similarly, readings made at five and fifteen minutes may reveal abnormal retention in cases in which later values may be normal. The usual practice is to make single determinations at thirty minutes with the 2 mg. dosage and at forty-five minutes with the 5 mg. dosage, the latter being the more popular. Greater sensitivity is attained by making serial determinations, establishing a curve of dye removal from the blood stream, but this is seldom resorted to in clinical practice. The rate of removal is somewhat more rapid after meals (increased hepatic blood flow) than in the fasting state. This fact emphasizes the necessity of performing this test under uniform conditions, preferably fasting, if results obtained at different times are to be compared.

In the doses employed, bromsulfalein is practically nontoxic, even in the presence of severe liver damage. The solution used is irritating to the tissues and may cause pain and inflammation if a significant amount is injected outside the vein. It may also produce thrombophlebitis at the site of injection. Occasional toxic reactions have been observed, including fever, nausea, vomiting, vertigo, and syncope. Allergic reactions have been reported, and also, in a few instances, severe anaphylactoid reactions, with serious cardiorespiratory manifestations and shock. Although these are extremely unusual, care should be exercised in performing this text in subjects with an allergic background.

BSP EXCRETION IN BILE. Studies of the curve of elimination of bromsulfalein in the bile (duodenal intubation) may yield valuable information in some cases in which the rate of removal of dye from the blood may be normal. Under normal conditions, with the 2 mg. dosage, the dye usually appears in the bile within five to fifteen minutes, its excretion continuing over a period of two to six hours. Fifty to 85 per cent of the quantity injected is excreted in the bile in one hour and 65 to 100 per cent in two hours, the curve of excretion reaching a maximum level at forty-five to seventy-five minutes and falling to a relatively low level at two hours. These findings suggest that the dye is removed rapidly from the blood and is subsequently excreted more slowly in the bile.

Abnormalities of the mechanism for removing bromsulfalein from the body may be evidenced by one or more of the following phenomena: (1) abnormal retention in the blood after injection; (2) delayed appearance of dye in the bile; (3) delayed attainment of maximum concentration in the bile; (4) prolonged high curve or low flat curve of biliary excretion of the dye; (5) subnormal total excretion in the bile at one or two hours after injection. In some cases, especially with incipient biliary obstruction or mild hepatic functional impairment, disturbances may occur in the "biliary" phase of excretion in the absence of abnormal dye retention in the blood. However, the clinical usefulness of this procedure is limited by the fact that duodenal intubation is required, and by certain technical difficulties.

Significance of Abnormal BSP Retention. Inasmuch as the hepatic excretory mechanisms for bilirubin and BSP are apparently similar, abnormal retention of both would be expected to occur in the same types of conditions, e.g., biliary obstruction and hepatocellular damage. This is, indeed, the case. However, it would appear that the test dose of BSP employed constitutes a greater functional burden on the excretory mechanism than does the amount of bilirubin formed in the course of normal degradation of hemoglobin. Consequently, abnormal BSP retention occurs practically invariably in the presence of hyperbilirubinemia, except, of course, that due to excessive hemolysis. The BSP test is therefore of no diagnostic value in patients with clinical jaundice (except hemolytic jaundice) or in those with abnormally high total or one-minute direct-reacting serum bilirubin concentrations. It should be noted, however, that BSP can be measured in jaundiced serum by proper photoelectric colorimetric procedures, and that the presence of jaundice or liver damage does not in itself contraindicate the use of this procedure. The BSP test is particularly useful in the study of patients with hepatic or biliary tract disease in whom the serum bilirubin concentrations are within normal limits.

Extrahepatic Biliary Obstruction. In the presence of obstruction of the common duct, the degree of retention of the dye increases progressively with increasing bilirubinemia until all of the dye injected remains in the blood at the end of the test period. In the presence of obstruction, therefore, no information is obtained by this method which cannot be obtained by means of the quantitative determination of serum bilirubin. Following relief of the obstruction, dye retention, although diminishing, frequently persists for a variable period of time after the serum bilirubin concentration has returned to normal. This is due perhaps to the residual liver damage which is present in nearly all patients who have suffered from biliary obstruction for an extended period, especially that due to biliary calculi.

Hepatocellular Damage. Abnormal retention of bromsulfalein in the blood occurs almost invariably in acute hepatic disease, the degree of retention being roughly indicative of the extent of hepatic functional impairment. This is true of all varieties of hepatitis and acute and subacute hepatic necrosis due to any cause. The degree of dye retention usually, but not invariably, parallels the degree of hyperbilirubinemia in such cases. As in the case of obstructive jaundice, abnormal dye retention may occur before and may persist longer than hyperbilirubinemia. Some observers believe, therefore, that this test is of value in the early detection of hepatocellular damage in patients receiving hepatotoxic agents for therapeutic purposes, such as arsphenamine and carbon tetrachloride.

Varying degrees of dye retention, up to 100 per cent, may be obtained in patients with chronic hepatic lesions. Among these are chronic hepatitis, portal cirrhosis, biliary cirrhosis, malaria, hepatic syphilis, carcinoma of the liver and chronic passive congestion of the liver. In many patients with hepatocellular damage, as in those with extrahepatic obstructive lesions, dye retention occurs only in the presence of hyperbilirubinemia, the degree of retention of dye and pigment being approximately parallel. Frequently, however, this relationship is not maintained. For example, a disproportionately high degree of dye retention may occur in patients with myocardial failure and passive congestion of the liver, as well as in those with hepatic functional impairment complicating thyrotoxicosis, pneumonia and severe anemia. This may be due, in part at least, to the effect of hepatic circulatory disturbance in contributing to decreased removal of BSP from the blood stream (p. 542). The majority of observers agree that in portal cirrhosis, particularly, dye retention may occur in the absence of hyperbilirubinemia. Under such circumstances this procedure is of great value from a diagnostic standpoint.

Investigation of the ability of the liver to eliminate dyes, therefore, is of greatest clinical value in cases of hepatic dysfunction in which frank hyperbilirubinemia is absent. Among such conditions are certain cases of portal cirrhosis, cholecystitis, cholelithiasis, toxemia of pregnancy, chronic hepatitis, syphilis and various acute infections, such as pneumonia and typhoid fever. It is often stated that dye retention occurs in cases of gallbladder disease with frank jaundice but not usually in those with latent or no icterus. In our experience, however, many patients with cholecystitis, with and without cholelithiasis, may show varying degrees of bromsulfalein retention, in the absence of, or with very slight degrees of hyperbilirubinemia.

In this connection, certain comments made regarding the significance of elevated direct-reacting serum bilirubin concentrations (p. 525) possibly apply also to BSP retention. Attention was called to the fact that total serum bilirubin values within the limits of normal, especially between 0.8 and 1.0 mg./100 cc., may in reality be abnormally high. It is in these cases particularly that the demonstration of abnormal BSP retention is of great value in revealing impairment of hepatic excretory function. As indicated above, this situation occurs frequently in patients with cirrhosis, infectious hepatitis (early and during convalescence), and cholecystitis.

Other Conditions. Abnormal BSP retention occurs in conditions in which the hepatic blood flow is diminished, with or without hepatocellular damage. These include congestive heart failure, and some cases of medical and traumatic shock. However, the hepatic blood flow is often essentially normal in shock and hemorrhage, in the absence of intrinsic liver damage, and the degree of BSP retention does not parallel the severity of shock or the amount of blood lost. In such conditions, impaired liver function may result from anoxemia of liver cells due to vasoconstriction (portal vein and hepatic artery branches).

Normal BSP values are strong evidence against ruptured esophageal varices (i.e., cirrhosis) as the cause of severe gastrointestinal bleeding. It must be recalled in this connection that hyperbilirubinemia and BSP retention may occur in patients with duodenal ulcer.

Slight, transient BSP retention may occur in the presence of fever, in the absence of demonstrable liver damage. It may occur also following operative procedures, particularly those involving the bile passages. This, too, is usually transient in nature.

Rose Bengal Excretion. In normal subjects, when 5 to 10 cc. of a 1 to 2 per cent solution of rose bengal are injected intravenously, the concentration of dye in a blood sample withdrawn eight minutes after injection of the dye should be not greater than 50 per cent of that in a sample withdrawn two minutes after injection. Abnormal retention of this dye has the same clinical significance as abnormal retention of bromsulfalein.

SERUM ALKALINE PHOSPHATASE ACTIVITY

The normal range of serum alkaline phosphatase activity is as follows: Bodansky method, 1.5 to 4 units/100 cc. in adults, 5 to 14 units in growing children (p. 249); King-Armstrong method, 3 to 14 units/100 cc. in adults; Jenner-Kay method, 0.1 to 0.2 units/cc. in adults, 0.17 to 0.34 units/cc. in children. Variations in this factor may occur in hepatic and biliary tract disease.

Serum phosphatase activity is almost invariably increased in the presence of jaundice due to mechanical obstruction of the bile ducts. Values as high as 100 units (Bodansky) have been reported in patients with obstructive jaundice. A notable exception is represented by the report of normal serum phosphatase activity in infants with congenital atresia of the bile ducts. Some believe that in obstructive jaundice serum phosphatase activity and serum bilirubin concentration increase approximately proportionately until the limit of phosphatase activity is reached (about 40 units). However, this is not invariably the case, occasional instances having been observed of simultaneous high phosphatase values and low serum bilirubin concentrations, and vice versa, in this condition. In cases of partial obstruction of the common bile duct (cholangitis, stone) or in intrahepatic duct obstruction (metastatic malignancy), an increase in serum phosphatase activity may occur with little or no increase in serum bilirubin concentration. A similar phenomenon has been observed in dogs following ligation of single bile ducts.

Increased serum phosphatase activity also occurs in clinical and experimental forms of hepatocellular jaundice. Values as high as 70 units (Bodansky) have been reported in arsphenamine hepatitis. This, in the case of arsphenamine, is attributed by some to intrahepatic bile duct obstruction, since, with the exception of toluenediamine, other hepatotoxic agents produce relatively slight or moderate increase in phosphatase activity in experimental animals in proportion to the degree of hyperbilirubinemia. In contrast to obstructive jaundice, values for serum phosphatase activity usually do not parallel the increase in serum bilirubin concentration in hepatocellular jaundice. Whereas high values are encountered occasionally in such cases, in the great majority of instances the serum phosphatase activity is normal or only slightly increased (usually less than 12 units). However, analyses of large groups of cases reveal the fact that no sharp distinction can be made between obstructive and hepatocellular types of jaundice on the basis of the presence or absence of parallelism between the increase in serum phosphatase activity and the rise in serum bilirubin concentration. Furthermore, although a distinctly larger proportion of patients with obstructive than of those with hepatocellular jaundice exhibit very high serum phosphatase values, the relatively wide overlapping of values in the two groups precludes the possibility of differentiating between them on this basis alone. In a study of 300 patients with major disorders of the liver and biliary tract, values above 10 units per 100 cc. were obtained in about 90 per cent of cases of obstruction of the common bile duct, in 18 per cent of cases of hepatocellular jaundice, in about 22 per cent of cases of advanced cirrhosis and in about 50 per cent of cases of liver abscess. No increase has been observed in cases of hemolytic jaundice, a fact which may be of value in differential diagnosis.

Serum phosphatase values ranging from normal to over 60 units (Bodansky) per 100 cc. have been observed in patients with portal cirrhosis with and without hyperbilirubinemia. In this condition, no parallelism exists between serum phosphatase activity and serum bilirubin concentration. The same is true of cases of metastatic carcinoma of the liver, in which condition a rather marked increase in alkaline phosphatase may occur in cases in which no significant increase in serum bilirubin concentration can be demonstrated. Values as high as 35 units per

100 cc. have been reported in such cases in the absence of skeletal metastatic lesions. Elevated values in patients with known extrahepatic malignancy, e.g., stomach, in the absence of jaundice or skeletal involvement, suggest the presence of liver metastases (p. 252).

The serum alkaline phosphatase activity is increased also in other forms of focal and diffuse liver disease, including abscess, sarcoidosis, echinococcus cyst, often in the absence of hyperbilirubinemia. An increase has been reported also following obstruction of the pancreatic ducts, in the absence of abnormality in the liver or bile ducts. It has been suggested, therefore, that complicating pancreatitis may contribute to the serum phosphatase increase in biliary tract disease.

The mechanism underlying these changes in serum alkaline phosphatase activity in hepatic and biliary tract disease is not well understood. Large amounts of phosphatase are excreted daily by the liver in the bile. In view of this fact, it has been suggested that the increase in serum phosphatase activity in jaundice of obstructive and hepatocellular origin may represent a retention phenomenon dependent upon obstruction to flow of bile, extrahepatic or intrahepatic. This view appears to be supported by histochemical studies of the distribution of alkaline phosphatase in the liver (dog) after ligation of the common duct as contrasted with that in livers of animals with liver damage. This hypothesis would appear to be contradicted by the following observations: (*a*) the occurrence in portal cirrhosis of marked increase in serum phosphatase activity, at times, with little or no increase in serum bilirubin, and vice versa; (*b*) normal phosphatase values in cases of congenital atresia of the bile ducts. However, the occurrence of increased phosphatase activity with little or no hyperbilirubinemia has been attributed to the fact that phosphatase is apparently not excreted by the kidneys. The absence of marked increase in phosphatase activity in hepatitis, despite marked hyperbilirubinemia, has been attributed to possible shunting of the enzyme around the damaged hepatic cells. It is difficult, however, to reconcile the normal values in infants with congenital atresia of the bile ducts with the "retention" theory of the cause of increased serum phosphatase activity in obstructive jaundice. Moreover, total hepatectomy (dog) is not followed by an increase comparable to that which follows common duct obstruction.

It is believed by some that the alkaline phosphatase which accumulates in the blood in bile duct obstruction and in certain types of hepatic disease originates in the liver cells or bile duct epithelium and, instead of being excreted in the bile, the normal route, enters the blood stream (via lymphatics or portal sinusoids). According to this view, the phosphatase occurring normally in the bile is not that originating in the skeleton and present in the blood stream. There is evidence that this skeletal phosphatase is not removed from the blood by the liver and excreted in the bile. On this basis, the absence of a significant increase in extensive hepatocellular damage is readily understood, i.e., failure of production. Increase in the serum in the absence of significant biliary obstruction or hyperbilirubinemia in such conditions as malignancy, sarcoidosis, cirrhosis, etc., would also be explicable as due to increased production by actively regenerating cells and proliferating bile ducts, which are rich in phosphatase, as are malignant cells. However, there is contradictory evidence regarding the identity of biliary, hepatic, and skeletal alkaline phosphatase, so this issue is far from settled.

Nevertheless, certain generalizations may be made concerning the interpretation of serum phosphatase activity in hepatic and biliary tract disease. Changes in this factor in other conditions are discussed elsewhere (p. 249 ff.).

1. High or rising values, above 15 Bodansky units, in patients with marked or increasing hyperbilirubinemia, suggest uncomplicated extrahepatic biliary obstruction. However, similar findings are common also in arsphenamine hepatitis, and in other conditions in which intrahepatic duct obstruction due to cholangiolitis is a dominant feature.

2. Normal or but slightly elevated values, below 10 Bodansky units, in patients with marked or increasing hyperbilirubinemia, suggest diffuse hepatocellular damage.

3. Values decreasing from elevated levels in patients with marked or increasing hyperbilirubinemia suggest increasing hepatocellular damage superimposed on bile duct obstruction.

4. Elevated values in the presence of normal serum bilirubin concentrations occur in portal cirrhosis, liver abscess, sarcoidosis, metastatic malignancy of the liver, obstruction of single hepatic ducts (e.g., by lymph nodes), histoplasmosis, echinococcus cyst, and pancreatic duct obstruction.

5. Cholangitis and cholangiolitis tend to produce an increase, and severe inanition a decrease in serum alkaline phosphatase activity. An increase occurs in the presence of active liver regeneration and bile duct proliferation.

6. Although the estimation of serum alkaline phosphatase activity may aid in differential diagnosis in some cases of jaundice, as indicated above, the value of this factor in this connection is limited by the fact that there is considerable overlapping of values in the obstructive and hepatocellular groups, only moderately elevated values (below 15 units) occurring in about 15 per cent of patients with extrahepatic duct obstruction, and values between 10 and 20 units in about the same proportion of patients with primary hepatocellular damage.

SERUM CHOLINESTERASE

The blood plasma contains an enzyme capable of splitting acetylcholine and certain other choline esters (nonspecific cholinesterase). This enzyme apparently originates largely in the liver, the blood levels falling in animals after hepatectomy or following administration of hepatotoxic agents. Activity is expressed in terms of change in acidity (pH) resulting from liberation of acetic acid from the acetylcholine substrate. Normal serum values range from 0.35 to 1.1 pH units according to some, and 0.7 to 1.4 pH units according to others.

In general, reasonably normal values are the rule in uncomplicated extrahepatic obstructive jaundice, and subnormal values are usually obtained in patients with severe hepatocellular jaundice, and also in advanced cirrhosis. It has been found that changes in the level of serum cholinesterase parallel those in serum albumin, both substances being proteins formed in the liver. The serum cholinesterase ctivity is influenced by conditions other than primary hepatic disease, including ver, inanition, thiamine deficiency, diabetes mellitus, and thyrotoxicosis. or these reasons, and also because of lack of agreement as to the range of normal alues, determination of this factor has not found wide acceptance in differential iagnosis.

BLOOD AMYLASE (DIASTASE)

Normal values for blood amylase (Somogyi) range from 60 to 180 units. Values below 60 have been obtained in dogs poisoned with carbon tetrachloride nd in patients with hepatic and biliary tract disease, including acute and chronic holecystitis, infectious and toxic hepatitis, cirrhosis, acute hepatic necrosis, hronic passive congestion of the liver, fatty liver, etc. Low values in diabetes mellitus, severe burns, thyrotoxicosis, toxemia of pregnancy, acute alcoholism, following surgical operations and in poisoning with chloroform, carbon tetrachloride, arsenic, and barbiturates have been attributed to hepatic damage. The ause of the fall in blood diastase in hepatic disease is not known, nor has its relation to the state of hepatic function, as determined by other methods, been ascertained.

High blood diastase levels have been reported in diseases of the liver and biliary tract. It seems probable that in the majority of such cases the increase is due to complicating acute pancreatic disease (p. 490) or to renal functional impairment.

PLASMA VITAMIN A AND CAROTENE (CAROTENOID)

The normal plasma vitamin A concentration ranges from 20 to 43 micrograms and for carotene 100 to 368 micrograms per 100 cc. Subnormal values for both vitamin A and carotenoid have been obtained in patients with acute hepatitis and cirrhosis of the liver. The lowest vitamin A values occur in decompensated cirrhosis and are probably related to the development of clinical manifestations of vitamin A deficiency in such cases. This may probably be due to faulty absorption or to fever, in part at least, but it is generally attributed to interference with the intermediary metabolism of vitamin A (inadequate conversion of carotene to vitamin A and inadequate storage of the latter in the liver) (p. 453). There is evidence, however, that the intestinal epithelium, rather than the liver, may be the site of synthesis of vitamin A from carotene. The vitamin A content of cirrhotic livers has been shown to be low. Although normal values are the rule in uncomplicated extrahepatic obstruction and subnormal values in hepatocellular damage, this procedure adds little information of value in the clinical study of patients with hepatic or biliary tract disease.

THE LIVER IN WATER AND SALT METABOLISM

In the presence of hepatic disease, disturbances may occur in the regulation of water, sodium, and chloride balance. Several mechanisms are undoubtedly involved. The nature of some of these is not well understood, but it seems desirable to review certain observations which have clinical implications.

The tendency of patients with hepatic disease, especially cirrhosis, to develop edema is well known. This is due in part, but by no means entirely, to the hypoalbuminemia which occurs in many of these cases (p. 505). Increase in plasma volume and interstitial fluid volume has been demonstrated in both acute and chronic liver disease (infectious hepatitis, cirrhosis), accompanied by oliguria and decrease in the amount and concentration of sodium and chloride in the urine, sweat, and saliva. Ingestion of a large volume of fluid results in much greater increase in extracellular fluid volume than occurs in normal subjects. The diuretic response to water (water tolerance test) is diminished, and intravenous injection of hypertonic sodium chloride solution does not produce the normal prompt augmentation of urine volume and sodium and chloride excretion, especially in cirrhotic patients with edema and/or ascites.

Inasmuch as the diminished renal clearance of sodium (and chloride) occurs in the presence of normal glomerular filtration rate and renal blood flow, the abnormal renal retention of sodium is attributed to its increased tubular reabsorption. A similar phenomenon occurs in congestive heart failure. As in the latter condition, clinical improvement in hepatic disease is often accompanied by spontaneous and copious diuresis.

These abnormalities in sodium and water excretion resemble those induced by excessive amounts of so-called salt-active adrenal hormones, estrogens, and progesterone. Increased amounts of corticoids and estrogens have been found in the urine of patients with hepatic disease, and it has been suggested that the retention of sodium and water may be due, in part at least, to inadequate removal of these hormones from the systemic circulation by the damaged liver (p. 315).

There have been reports of the presence of increased amounts of an antidiuretic principle in the urine of subjects with hepatic disease. According to some, this may be the antidiuretic hormone of the posterior pituitary, the assumption being that it is either produced in excessive amounts or inadequately destroyed by the liver. The data are contradictory and opinion is divided on this point. The possibility has been suggested that the water retention in these cases may be due to the presence in the blood of excessive amounts of the hepatic vasodepressor factor (VDM) (apoferritin), which induces antidiuresis under certain circumstances, presumably by stimulating discharge of antidiuretic hormone by the posterior pituitary. It has been shown that this substance, produced in the liver, undergoes prompt inactivation in that organ normally, but not under certain abnormal conditions, e.g., anoxia. This question, too, is as yet unsettled.

LIVER FUNCTION STUDIES IN DIFFERENTIAL DIAGNOSIS

The most important difficulties encountered in the clinical employment of tests of liver function arise from attempts to interpret functional findings too strictly in terms of disease diagnosis. The fact is too frequently overlooked that tests of function, if they accomplish their purpose, indicate the state of functional activity of an organ and not necessarily the presence or extent of morphologic changes in that organ. This is particularly true of the liver, because of its enormous regenerative capacity and large functional reserve (p. 495). In the case of this, as of other organs, a sharp distinction must be drawn between disease diagnosis and functional diagnosis. However, function tests, used properly, may aid greatly in establishing a disease diagnosis, complementing information obtained by other methods.

The diagnosis of uncomplicated hemolytic jaundice usually offers little difficulty. Apart from the physical and hematological findings, there are characteristically (*a*) hyperbilirubinemia with a negative direct van den Bergh reaction, (*b*) little or no increase in direct-reacting serum bilirubin, (*c*) usually no bilirubin in the urine, (*d*) increase in urobilinogen in the urine and feces, (*e*) no bile salts in the urine, (*f*) hypocholesterolemia, and (*g*) normal findings by other tests of liver function (dye excretion, hippuric acid synthesis, phosphatase activity, prothrombin response to vitamin K, globulin reactions, etc.). In a fairly large proportion of cases of congenital hemolytic jaundice and sickle-cell anemia this characteristic picture is complicated by the occurrence of common-duct obstruction by gallstones, with the development of manifestations of obstructive jaundice, which may dominate the clinical picture.

Difficulty is frequently encountered in differentiating between *hepatocellular* and *extrahepatic obstructive* jaundice, and it is chiefly in this connection that attempts are made to utilize function tests as an aid in disease diagnosis. For this purpose it is advisable to group these tests in two categories: (1) tests of "metabolic functions," which depend entirely upon the functional integrity of the liver cells and are uninfluenced by interference with the flow of bile, unless this produces hepatocellular damage; (2) tests of "excretory function," which depend upon maintenance of a free flow of bile as well as upon hepatocellular function. The most important of the "excretory tests" are the serum bilirubin concentration and the dye excretion tests. The "metabolic tests" include the great majority of the other procedures that have been discussed in connection with the estimation of hepatocellular function. In this category are included also the so-called "globulin reactions" (thymol turbidity, cephalin-cholesterol flocculation, etc.), which are not in reality tests of liver function (p. 508). In fact, their chief value in differential diagnosis arises out of the fact that they appear to reflect the presence and extent of hepatocellular damage (in hepatic disease) rather than the state of liver function.

In studying patients with jaundice, the results of the "excretory tests" should be weighed against those of the "metabolic tests." The combination of severe hyperbilirubinemia and marked bromsulfalein retention with normal hippuric acid synthesis, serum phosphatase activity, cholesterol and cholesterol ester concentration, prothrombin response to vitamin K, negative cephalin-cholesterol flocculation and thymol turbidity reactions, etc., indicates serious interference with removal of bromsulfalein and bilirubin from the blood but no disturbance of the other functions investigated. This state of affairs is explicable only on the basis of extrahepatic bile duct obstruction with little or no significant hepatocellular damage. However, this hypothetical situation is rarely encountered clinically except in very early stages of obstruction of the common duct due to tumor (pancreas, duodenum, bile duct, lymph nodes). Before very long, even in such cases, there develops a variable degree of hepatocellular damage due to increased intraductal pressure or associated factors, and this damage may be reflected in impairment of the "metabolic" functions of the liver. Except in cases of early, partial obstruction, therefore, there is usually some degree of abnormality of one or more of the tests of hepatocellular function ("metabolic tests"). Under such circumstances, evaluation of the relative degree of impairment of the "metabolic" and "excretory" functions may be helpful in differential diagnosis, the latter often being impaired relatively more than the former in primarily obstructive jaundice. Illustrative data in this connection are presented in Table 39. However, moderate or severe impairment in both groups of tests, indicating moderate or severe impairment of hepatocellular function and probably extensive hepatocellular damage, does not by any means exclude the possible presence also of extrahepatic bile duct obstruction. It must be realized that primary hepatocellular damage and that superimposed on extrahepatic duct obstruction may produce essentially the same results with most of the so-called "metabolic tests," insofar as liver damage is reflected in impaired function. From the standpoint of differential diagnosis of the cause of jaundice, therefore, the most definitive findings are obtained in cases of uncomplicated extrahepatic

Table 39. Degree of Impairment of Metabolic as Compared to Excretory Functions of the Liver in Patients with Hepatocellular Damage and Extrahepatic Biliary Obstruction

CONDITION	EXCRETORY TESTS	METABOLIC TESTS			
		Normal	Slight	Moderate	Severe
		% of total cases			
Acute hepatitis	Normal	10	78	12	0
	Slight	0	65	30	5
	Moderate	0	7	46	47
	Severe	0	0	38	62
Cirrhosis	Normal	13	72	15	0
	Slight	0	29	71	0
	Moderate	0	20	67	13
	Severe	0	0	51	49
Stone in common duct	Normal	0	0	0	0
	Slight	38	34	28	0
	Moderate	20	44	18	18
	Severe	0	6	58	36
Carcinoma of pancreas	Normal	0	0	0	0
	Slight	78	12	10	0
	Moderate	34	30	18	18
	Severe	18	24	24	34

duct obstruction, e.g., neoplastic. Equivocal results are frequently obtained in the much more common cases of calculous obstruction, which is often accompanied by hepatocellular damage.

Duodenal intubation and study of the bile are useful in the diagnosis of the nature of a biliary tract lesion (pigmented bile duct and gallbladder epithelium, leukocytes, red blood cells and cholesterol and calcium bilirubinate crystals). In a patient with jaundice, failure to obtain bile after prolonged and repeated duodenal intubation and stimulation is suggestive of complete obstruction of the bile duct. This diagnosis is supported by the absence of urobilinogen from the urine and feces (p. 530).

In the ultimate analysis, accurate diagnosis in lesions of the liver and bile passages depends primarily upon the clinical history, physical examination, x-ray studies, liver biopsy, and examination of the bile, supplemented by carefully interpreted functional findings. The last must not be accorded undue imp ortance in establishing a disease diagnosis, although they may be very helpful undercertain circumstances. Perhaps the most important contribution to hepatic disease diagnosis has been the increasing use of liver biopsy as a routine procedure. On the other hand, these studies have served to emphasize the frequent lack of correlation between morphologic and functional findings. In diffuse hepatocellular damage, morphologic changes are usually accompanied most consistently by abnormalities in BSP excretion, serum albumin and globulins, and globulin reactions (thymol turbidity and flocculation, zinc sulfate turbidity, cephalin-cholesterol flocculation). As has been pointed out, however, increase in γ-globulin, which is the most common globulin abnormality in these conditions, cannot properly be regarded as a reflection of impaired liver function, and results obtained with "globulin reactions" should not be included in an evaluation of the significance of liver function tests in differential diagnosis. Whereas significant liver damage may be present in the absence of demonstrable abnormality of function, as measured by available procedures, the reverse may also occur, i.e., evidence of functional disturbance in the absence of significant demonstrable morphologic change. Moreover, the degree of impairment of various functions frequently cannot be predicted on the basis of the nature or extent of the observed morphologic abnormalities.

The fact must be borne in mind that there is considerable variation in the sensitivity of the many functions of the liver to noxious influences, as well as in the sensitivity of the test procedures available. In the presence of mild functional impairment, significantly abnormal results may be obtained with only one or a few of the tests employed, emphasizing the necessity of applying as many of the available procedures as possible in all cases. In view of the fact that the liver is performing hundreds of chemical reactions, and is involved in excretory as well as purely metabolic processes, it is obvious that its functional activity cannot possibly be measured by a single test. Moreover, it should be obvious that hope for a function test that will clearly differentiate obstructive from hepatocellular jaundice, i.e., one that will make a disease diagnosis, is not founded on reality.

It should be realized, too, that serial determinations are much more valuable than single observations. The earlier these are begun in the course of the disease, the more information they provide. For example, similar findings may be obtained in patients with hepatitis and those with calculous obstruction of the common duct and superimposed infection of the duct system and hepatocellular damage. However, in the latter, studies performed earlier, before the development of extensive hepatic disease, might have suggested the primarily obstructive nature of the jaundice. Apart from their usefulness in differential diagnosis, serial determinations are invaluable in following the progress of such diseases as infectious hepatitis, hepatic necrosis, and cirrhosis.

The question is often raised as to what tests are most useful. The fact is too frequently overlooked that selection of the most valuable procedures in a given case is determined to a large degree by the type of information desired. In the presence of clinical jaundice, the problem is primarily that of establishing its nature, i.e., hemolytic, hepatocellular, or extrahepatic obstructive. The BSP test is of no value in this connection except in the diagnosis of hemolytic jaundice, which can usually be made readily by other means. Consistent absence of urobilinogen in feces and urine suggests complete and permanent, i.e., usually neoplastic (or stricture), obstruction. Otherwise, urobilinogen determinations are of limited value in distinguishing between so-called medical (e.g., hepatitis) and surgical (e.g., calculous obstruction) forms of jaundice. Serum albumin and globulin determinations, and the globulin reactions may be very helpful, and should be performed routinely. Prothrombin determinations are indicated in all such cases, because of the possibility of requiring surgical intervention. Consequently, it is advisable to make determinations, as a routine, before and after administration of vitamin K; at times this procedure aids in differential diagnosis. In still doubtful cases, determination of serum alkaline phosphatase activity, plasma cholesterol concentration and partition, and hippuric acid synthesis should be made, in the order named. If the issue is still undecided, in our experience, no additional information can be gained from other liver function tests.

In the presence of known calculous obstruction, with jaundice, a different problem exists. The obstruction is usually incomplete; the serum bilirubin concentration should be determined at intervals to ascertain whether bile stasis is increasing or decreasing. Before intervening surgically, in addition to investigating the plasma prothrombin, information concerning the possible presence of superimposed liver damage and the state of liver function is desirable. Serum albumin and globulin determinations and the globulin reactions should be performed routinely. If these are abnormal, no additional tests are required, if extrahepatic causes can be eliminated. Otherwise, determinations should be made of hippuric acid synthesis and plasma cholesterol concentration and partition.

In the presence of known acute diffuse hepatic disease, e.g., infectious hepatitis, no additional diagnostic problem is involved. Information is desired concerning the severity and progress of the disease. The severity can be gauged satisfactorily by the level of serum bilirubin, the degree of BSP retention, the lowering of the serum albumin and rise in globulin concentration, and the degree of abnormality of the globulin reactions. These tests should be performed as early as possible and the serum bilirubin concentration should be repeated at intervals; this usually suffices to indicate the progress of the disease. During convalescence, when the serum bilirubin has fallen to within normal limits, the subsequent course should be followed by periodic determinations of one-minute direct-reacting serum bilirubin, BSP retention, thymol turbidity, zinc sulfate turbidity, cephalin-cholesterol flocculation, and urine urobilinogen, eliminating each procedure when it yields normal results.

In the absence of clinical jaundice, the problem is one of the detection of possible hepatic functional impairment, at times for the purpose of diagnosis of otherwise unrecognizable liver disease, in other instances to follow the course of a known disease, e.g., cirrhosis. Since the presence of latent jaundice can be excluded only by determination of the serum bilirubin concentration, this must be done in all cases. If it is within normal limits, the one-minute direct-reacting bilirubin should be determined, and the BSP test performed. If these are normal the urine urobilinogen should be measured. The serum albumin and globulin should be determined and the globulin tests performed. In our experience, if these procedures yield normal results, little if any additional information is gained from others, with the possible exception of the hippuric acid synthesis test. In patients with known cirrhosis, progress of the disease can usually be followed satisfactorily by periodic determinations of serum bilirubin, albumin and globulin concentrations, the globulin reactions, and BSP retention. Attention should be called to the fact that, at least in infectious hepatitis, bilirubinuria may occur before other abnormalities can be demonstrated.

In interpreting abnormalities in such factors as the plasma proteins, plasma cholesterol, and alkaline phosphatase activity, and abnormalities in thymol and zinc sulfate turbidity and cephalin-cholesterol flocculation, the fact must be kept in mind that changes identical with those present in liver disease may be produced by extrahepatic lesions.

The usefulness of liver function tests in clinical practice may be summarized as follows:

1. The diagnosis of uncomplicated hemolytic jaundice can be established rather readily.

2. They aid materially in the diagnosis of uncomplicated extrahepatic duct obstruction.

3. They may reveal the presence of liver damage in patients with known obstructive lesions, e.g., stone, stricture, neoplasm.

4. Serial studies are of great value in following the course of acute diffuse liver disease, e.g., infectious and toxic hepatitis and hepatic necrosis. In these conditions, they are particularly useful in revealing residual damage after subsidence of other clinical manifestations, and in suggesting possible progression to chronic liver disease.

5. Certain tests may be of quite specific diagnostic value, e.g., Type III porphyrinuria in "alcoholic" cirrhosis, increased alkaline phosphatase (in the

absence of other demonstrable abnormalities) in metastatic malignancy of the liver and in liver abscess.

6. They may indicate the presence of unsuspected liver disease, e.g., cirrhosis.

7. They afford the only means of demonstrating the integrity or of evaluating the degree of impairment of various functions of the liver in the presence or absence of evidence of morphologic damage.

8. The "globulin reactions" should not be regarded as tests of liver function. They frequently reflect the presence and severity of liver cell damage, inflammatory or degenerative, and are therefore particularly useful in distinguishing between hepatocellular and extrahepatic obstructive types of jaundice and in following the progress of patients with acute diffuse hepatic diseases, particularly during their clinical subsidence.

CHEMICAL EXAMINATION OF BILE

The chemical examination of bile is not often resorted to clinically. However, certain changes which may occur under abnormal conditions are of distinct practical significance. Because of the fact that these changes are usually of a quantitative rather than a qualitative nature, bile obtained by duodenal intubation is seldom satisfactory for such studies because of its admixture with gastric, pancreatic and duodenal secretions. However, even under such circumstances, certain findings are of clinical importance. The most satisfactory and significant studies are those made on bile draining externally, usually through a tube placed in the common bile duct following operation on the bile passages. It is well recognized that, during its sojourn in the gallbladder, bile is concentrated to one-third to one-half of its original volume. The relative proportions of the dissolved solids may also be somewhat altered, particularly in cholecystitis, due to varying degrees of absorption of certain of these constituents from the gallbladder. The extreme rapidity with which water is absorbed by the gallbladder wall is indicated by the fact that the mere passage of bile through the gallbladder may diminish its volume by 50 to 75 per cent. The subsequent discussion will be restricted largely to observations made upon bile obtained by common-duct intubation.

Bile Acids. Bile acids, perhaps the most characteristic constituent of bile, occur in human bile chiefly as salts of the cholic and desoxycholic acid series and their derivatives. They are specific products of hepatic-cell activity and occur in human bile chiefly as conjugate amino acids, being combined with glycine and taurine (p. 535). Under normal conditions, 85 to 90 per cent of the bile acids entering the intestine is reabsorbed, only 10 to 15 per cent escaping in the feces. In patients with diarrhea the loss in the feces may be greater.

A decrease in the bile acid content of the bile has been observed in clinical states of extensive hepatic damage, experimentally following the administration of hepatotoxic agents, and following prolonged periods of total bile stasis. They have been found to be absent from the bile in patients in whom complete biliary obstruction has been present for a week or longer. The interval between the time of release of obstruction and the reappearance of bile acids in the hepatic bile is apparently roughly proportional to the degree and duration of obstruction and to the degree of associated cholangitis, being in some instances as long as twenty-seven days. This observation has a distinct bearing on prognosis following operation in such cases, since it indicates the extent of impairment of liver function. However, it has been suggested that these findings may be due to the prevalence of bile acids which do not respond to the Pettenkofer reaction during the first days of resumption of chologenesis (p. 537), as the colorimetric test yields figures which lag behind those obtained by gravimetric methods in some cases. It is interesting also that a decrease in the bile acid concentration of gallbladder bile has been found in normal pregnant women, but no studies are available regarding its concentration in the liver bile during pregnancy. Under normal conditions, the bile acid concentration of human liver bile has been found to range from 200 to 2000 mg. per 100 cc.

Lipids. Normal bile contains fatty acids, neutral fat, phosphatides and cholesterol, the last being by far the most important of these lipids because of its relation to the formation of gallstones. The cholesterol concentration of normal human liver bile ranges from about 20 to 200 mg. per 100 cc., usually being well below its concentration in the blood plasma. Whereas 60 to 80 per cent of the plasma cholesterol is in the form of esters, in the great majority of cases studied all of the bile cholesterol was in the free state. Indeed, it seems questionable whether cholesterol esters are present normally in liver bile unless it is contaminated with blood.

Studies of the effect of diet upon the cholesterol content of liver bile has been largely confined, for obvious reasons, to experimental animals. Caution must be exercised in applying such observations to human beings, owing to fundamental differences in the physiology of the biliary system in different species. However, certain of these observations may perhaps be applied clinically. It has been found that although the total output of cholesterol may possibly be influenced by dietary factors, its concentration in the bile remains practically unaltered even after feeding enormous amounts. Moreover, there appears to be no consistent relationship between the level of plasma cholesterol and its concentration in the bile. It is possible to raise the blood cholesterol without a corresponding increase in bile cholesterol and also to increase the cholesterol elimination in the bile without any alteration in the blood cholesterol concentration. An increase in the cholesterol content of both hepatic and gallbladder bile occurs in the late months of pregnancy and persists until shortly after birth of the child.

The lack of relationship between plasma and bile cholesterol is emphasized by the fact that in some cases of the nephrotic syndrome and diabetes mellitus with extremely high plasma cholesterol values, the bile cholesterol concentration may be low, while in some cases of pernicious anemia, with a relatively low plasma cholesterol concentration, the level in the bile may actually exceed that in the blood. Present views regarding the hepatic origin of the biliary cholesterol are discussed elsewhere (p. 100). The cholesterol content of liver bile is diminished in the presence of severe hepatic disease as well as during and following the relief of total bile stasis. Values of less than 10 mg. per 100 cc. of bile have been observed under certain circumstances, the degree of diminution and the interval elapsing before a return to normal being dependent apparently upon the extent of liver damage.

Inorganic Elements. The molar concentration of inorganic ions in hepatic bile is approximately the same as in blood serum. The one exception is magnesium which, according to some observers, is present only in traces in liver bile (0.5 mg. per 100 cc. in the dog). From a clinical standpoint, interest is centered particularly in changes which have been observed in the chloride concentration following release of biliary obstruction. It has been found that the chloride concentration of hepatic bile is usually higher than that of the blood plasma immediately after release of the obstruction. Persistence of this high level or a continued increase has been found to be of serious prognostic significance, while recovery is accompanied by a return of the bile chloride concentration to normal levels. These changes are consistent enough to be of some clinical significance in prognosis. The calcium content of hepatic bile immediately after release of biliary obstruction appears to be somewhat lower than normal.

Miscellaneous Constituents. Bile normally contains mucoprotein, ammonia, urea, purine derivatives and amino acids. Leucine and tyrosine appear in the bile in cases of severe hepatic necrosis. Relatively small amounts of glucose are also present in the fasting state, increasing after the ingestion of foods and in abnormal hyperglycemic states. The usual absence of glucose from gallbladder bile is attributed to rapid glycolysis in that viscus. Only traces of amylase and no lipolytic or proteolytic enzymes are usually present. The only enzyme of significance present in abundance in hepatic bile is phosphatase (p. 248). Bile also constitutes an excretory medium for many substances, including lead, copper, bismuth, arsenic, mercury, iron, iodine, sulfonamides, salicylates, etc.

It has been found that large amounts of exogenous and endogenous estrogens are excreted in the bile. There is evidence that these undergo an enterohepatic circulation similar to that of bile acids. Vitamin D is excreted in the bile and androgens have also been found. It seems probable that the bile may be an important medium of excretion of other steroid compounds.

Bile Pigment. Bilirubin and biliverdin are the most important pigments of normal human bile, the latter being an oxidation product of the former. The pigment of freshly secreted human bile consists practically entirely of bilirubin. Its concentration varies considerably from time to time, the range of values being 50 to 170 mg. per 100 cc. It has been found that in biliary obstruction there is a diminution in the amount of pigment in the bile, this change being masked at times by the concentrating action of the gallbladder. With obstruction of relatively brief duration there is a rapid increase in both concentration and total output of bile pigment during the greater part of the first week of decompression, with a subsequent decrease to approximately normal levels. If obstruction has been present long enough to produce permanent hepatic damage, or in the presence of hepatic dysfunction due to other cause, there is a decrease in both the concentration and the total output of bile pigment, frequently with the excretion of relatively large quantities of pale fluid with a low total solid concentration.

White Bile. When any portion of a damaged extrahepatic biliary conducting system becomes obstructed, the static bile is gradually diluted by the mucoid secretion of the duct epithelium and eventually becomes colorless, the bile pigment being either absorbed or transformed into a colorless substance. This "white bile" is important because of its great prognostic significance when found in the common bile duct. The mechanism of its production has been the subject of considerable investigation. Recent studies indicate that "white bile" obtained from the gallbladder contains chloride and, as a rule, calcium at approximately the blood serum levels, the calcium being somewhat more variable than the chloride. The cholesterol concentration is very low and bile acids are absent, bile pigment being present in extremely small amounts or being entirely absent. The consensus is that "white bile" can be formed only if the hepatic parenchyma is no longer functioning normally and that it is largely, if not entirely, a product of the activity of the mucosal cells of the biliary conducting system.

Gallstones. Gallstones, in man, usually consist of cholesterol, either alone or with admixtures of calcium bilirubinate, carbonate or phosphate. Pure pigment stones are found only occasionally. Gallstones may form whenever the concentration of cholesterol in the bile exceeds its saturation point. This may happen because of an increase in the concentration of cholesterol or a decrease in its solubility in the bile. The quantitative relationship between bile salts and cholesterol in the bile may be important in preserving the state of solution of the latter under normal conditions and in determining precipitation in the presence of disease of the bile passages (p. 536). Decreased solubility of cholesterol in the bile may be due to the following causes: (*a*) during stasis in the gallbladder, the pH of bile is lowered and the faculty of bile acids for "complex" formation is diminished, with a diminution also in their dissolving power; (*b*) resorption of water by the gallbladder mucosa raises the concentration of all constituents of the bile, which brings most of the bile components to the limit of their saturation and leads to precipitation; (*c*) bile acids may be reabsorbed through an inflamed gallbladder mucosa so rapidly that the bile acid: cholesterol ratio falls to the point where cholesterol is precipitated. The disturbance in the ratio may be aggravated by the fact that the inflamed gallbladder mucosa may add relatively large quantities of cholesterol to the contained bile. This may be due either to active excretion or to desquamation of cholesterol-containing mucosal cells.

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CHAPTER XXII

Renal Function

As stated by Homer Smith, the composition of the blood plasma is determined by what the kidneys keep rather than by what the mouth ingests. These organs are the chief ultimate regulators of the internal environment of the body, and the urine is a by-product of their regulatory activities.

In accomplishing their purpose, i.e., maintenance of a reasonable constancy of composition of the extracellular and, to a certain extent, the intracellular fluids, the kidneys are involved in:

1. Elimination of water formed in or introduced into the body in excess of the amount required.
2. Elimination of nonvolatile end products of metabolism.
3. Elimination of inorganic elements in accordance with the needs of the organism.
4. Elimination of certain foreign substances that gain access to the body.
5. Retention in the body of substances necessary for the maintenance of normal functions, e.g., plasma proteins, glucose, amino acids, hormones, vitamins, etc.
6. Formation and excretion of certain substances, e.g., ammonia and hydrogen ions.

As a result of these activities the kidneys play an important part in:

1. The regulation of water balance (p. 296), electrolyte balance (p. 275), and osmotic pressure of the body fluids (p. 301).
2. The regulation of acid-base balance (p. 329).
3. The removal of metabolic waste products and certain toxic substances.

Morphological Features of Functional Importance. The nephron (uriniferous tubule) is the functional unit, each kidney containing about 1,200,000 of these structures. The nephron consists of two functionally distinct units, (*a*) the glomerulus, primarily a vascular channel, and (*b*) the tubule, lined by epithelial cells of different types in different portions.

After entering the kidney, the renal artery divides, successively, into interlobar, arcuate, and interlobular branches, the latter eventuating in the afferent arterioles of the glomeruli. The afferent arteriole breaks up into a set of capillary loops, each of which is enveloped in and intimately bound to the external (basement) layer of Bowman's capsule, the invaginated glomerular end of the tubule, lined by flattened epithelial cells that are apparently functionless. The attached walls of the glomerular capillaries and of Bowman's capsule together form a semi-permeable membrane, about 1 μ thick, across which substances pass from the blood plasma into the lumen of the tubule by a process of simple filtration.

The glomerular capillaries reunite to form the efferent arteriole of the glomerulus, the diameter of which is approximately one-half that of the afferent arteriole. This difference in caliber serves to maintain an effective "head" of filtration pressure in the capillaries under varying conditions of arteriolar tonus. The efferent arterioles pass to the several portions of the tubule, breaking up into an elaborate network of capillaries which lie in close approximation to the outer tubular lining, i.e., basement membrane of the tubular epithelial cells. They subsequently unite to form a venous plexus which leads, successively, into the interlobular, arcuate, and interlobar veins, and, finally, the renal vein.

These vascular features have an important bearing on certain important functional considerations, normal and abnormal. By far the bulk of the blood supplying the tubules passes first through the glomeruli, as indicated above. Consequently, interference with blood flow through glomeruli, e.g., in glomerulonephritis, is followed by degenerative changes (ischemia, anoxia) in the tubular epithelial cells supplied by the corresponding efferent arterioles. This accounts for the almost invariable secondary occurrence of nephrotic lesions as a complication of primary glomerular damage, unless of brief duration. Moreover, the blood plasma reaching the tubules has an increased colloid osmotic pressure owing to the fact that, in passing through the glomerulus, it has lost about 18 to 20 per cent of its water and diffusible components, but virtually no protein. The resulting increased viscosity of the blood (hemoconcentration) helps to maintain the relatively high pressure in the glomerular capillaries, and the increased plasma colloid osmotic pressure contributes to the extensive reabsorption processes that occur in the tubule.

In passing through the glomeruli, the blood loses an essentially protein-free plasma filtrate. In the course of its subsequent passage through the tubules, this filtrate is modified extensively by reabsorptive, excretory, and other activities of the tubular epithelial cells, the final product being urine. Largely as a result of the development of the clearance concept, various phases of renal function can be studied by rather precise quantitative methods. These are of value, not only in furthering understanding of the pathological physiology of certain disease states, but also in differential diagnosis, prognosis, and treatment of diseases of the kidneys or other conditions affecting renal function.

GLOMERULAR FILTRATION

Studies of glomerular fluid and serum filtrate (protein-free) have shown that the pH, vapor pressure, conductivity, and concentrations of urea, glucose, chloride, inorganic phosphorus, exogenous uric acid, exogenous creatinine, bicarbonate, etc. in these fluids are practically identical within the error of the methods employed. These observations indicate that the glomerulus acts merely as an ultrafilter and that glomerular urine is formed by a process of filtration alone, i.e., a purely mechanical process. The effective filtration pressure is the resultant of the blood pressure in the glomerular capillaries and the opposing forces of the colloid osmotic pressure of the blood plasma and the tension within Bowman's capsule (capsular pressure). The mean glomerular pressure may be regarded as about 50 to 70 per cent of the mean systemic arterial pressure (90 mm. Hg), thus averaging about 45 to 65 mm. Hg. This is subject to regulation by variation in the relative degree of constriction of the afferent and efferent arterioles of the glomerulus. Under stimulation, the latter may be constricted more than the former, with consequent increase in the pressure within the glomerular capillaries. If it is assumed that the colloid osmotic pressure is 24 mm. Hg in the blood entering the glomerulus, and that the capsular pressure is about 15 mm. Hg, it is obvious that the initial effective filtration pressure may range from 45 minus (24 + 15) or 6 mm. Hg to 65 minus (24 + 15) or 24 mm. Hg. As fluid (and diffusible solids, but little or no protein) leaves the plasma within the capillaries as a result of this force, the plasma protein concentration increases and may reach a point where the effective filtration pressure is so reduced as to impair glomerular filtration. Further constriction of the efferent arteriole may then still increase the glomerular blood pressure, allowing filtration to continue. In addition to the influence of these pressure factors, the volume of glomerular filtrate is influenced significantly by (*a*) the surface area of the filter (glomerular capillary surface, normally 1.56 sq. meters) and (*b*) the minute volume flow of blood plasma over this surface (normally about 700 cc. plasma or 1200 cc. blood per 1.73 sq. meters body surface). Under normal conditions, the volume of glomerular filtrate averages about 125 cc. per minute. This is discussed in detail in connection with the discussion of inulin clearance (p. 581).

It is apparent that glomerular filtration may be diminished with a tendency toward retention of waste products in the blood, in the absence of primary morphologic changes in the kidneys, by (1) extrarenal factors which diminish the renal blood flow and (2) factors which lower the effective filtration pressure by (*a*) decreasing the glomerular blood pressure, (*b*) increasing the plasma oncotic pressure (p. 584) or (*c*) increasing the capsular pressure. Among the most important of these are clinical conditions characterized by marked lowering of the systemic blood pressure, dehydration or hemoconcentration and cardiac failure (coronary artery occlusion), duodenal and pyloric obstruction, peritonitis, severe hemorrhage, Addison's disease, pneumonia, severe diarrhea, acute pancreatitis, perforated peptic ulcer or gallbladder, diabetic coma, extensive burns, left ventricular failure, urinary tract obstruction and various clinical states of shock. In such conditions, as in any condition of renal functional impairment, the tendency toward nitrogen retention may be aggravated by a simultaneous increase in protein catabolism or absorption of excessive amounts of protein end-products into the organism (e.g., in massive gastrointestinal hemorrhage).

In shock, both glomerular filtration and renal blood flow have been found to be decreased, approximately in proportion to the degree of shock. This decrease, which is greater than can be explained solely on the basis of the fall in blood pressure, results from active renal arteriolar constriction. Renal vasoconstriction plays a homeostatic role in shock, tending to maintain circulatory efficiency, for normally about 25 per cent of the resting cardiac output perfuses the kidneys. This, however, is effected at the expense of renal function, for oliguria and diminution in concentrating power result from the decreased renal circulation.

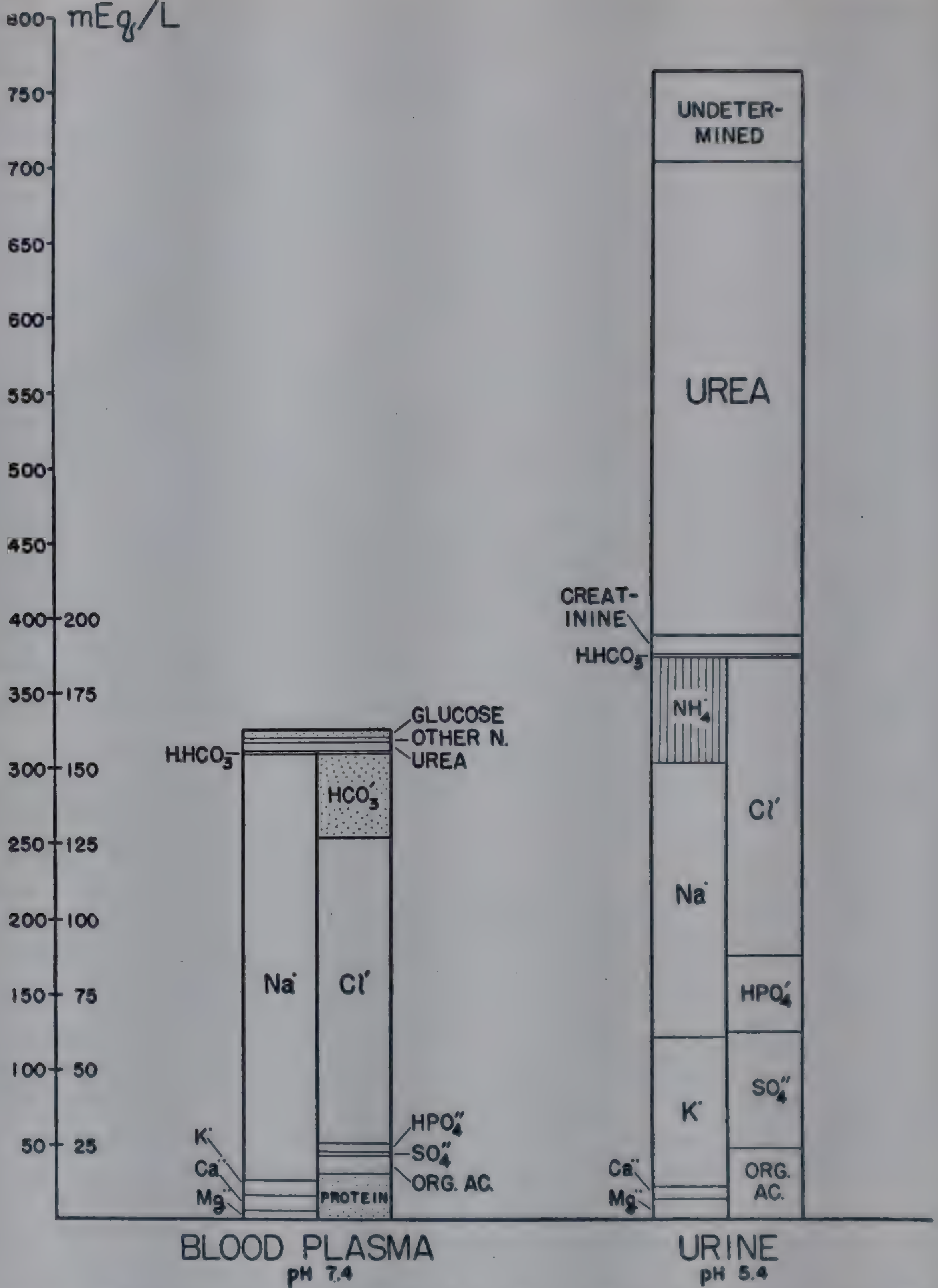

Fig. 53. Illustrating the manner in which the kidney defends the chemical pattern of the blood plasma, producing a substance (urine) differing widely and variably from blood plasma as regards the relative quantities of substances, osmotic value and reaction. The figures to the left (ordinate) indicate the sum of the milliequivalents of acid and basic ions. (After Gamble.)

TUBULAR FUNCTION

Reabsorption. If, as has been estimated, the rate of formation of glomerular urine under normal conditions is approximately 125 cc. per minute, while the rate at which urine passes into the bladder under the same conditions is approximately 1 to 2 cc. per minute, it is obvious that in its passage through the uriniferous tubules about 99 per cent of the water of the glomerular filtrate must have been reabsorbed. Furthermore, since the glomerular filtrate contains glucose in practically the same concentration as the blood plasma, whereas the bladder urine contains none, or very little, this substance, too, must have undergone practically complete reabsorption in the tubules. Quantitative studies of the excretion of other solids, such as chloride, phosphate, urea, uric acid, sodium, potassium, etc., in glomerular and bladder urine indicate a variable degree of reabsorption during their passage through the uriniferous tubules. Observations made in experimental animals have thrown considerable light upon the site of reabsorption of several of these solids in the renal tubules. It was found (in amphibians) that reabsorption of glucose occurs entirely in the proximal segment, reabsorption of chloride and sodium throughout the tubule, and acidification of the urine in the distal segment. The proximal tubule appeared to be capable of actively reabsorbing phosphate.

Insofar as excretory functions are concerned, therefore, the function of the renal glomeruli may be regarded as that of ultrafiltration (protein-free filtrate of the blood plasma) and that of the tubular epithelium, in part at least, as "selective" reabsorption (water, glucose, chloride, etc.). Inasmuch as the average rate of glomerular filtration is 120 to 130 cc. per minute (about 70 cc. per sq. meter of body surface) (p. 582), it follows that more than 170 liters are filtered through the glomeruli from the plasma daily, the tubules subsequently reabsorbing about 168.5 liters of water, 1000 gm. NaCl, 360 gm. $NaHCO_3$, 170 gm. glucose and smaller amounts of phosphate, sulfate, amino acids, urea, urate, etc., in order to excrete about 60 gm. of NaCl, urea and other waste products in about 1500 cc. of urine.

Several of the factors involved in the regulation of tubular reabsorption of various components of the glomerular filtrate are not entirely understood. However, information is available concerning certain constituents which aids materially in understanding abnormalities in disease. The extent to which any component of the glomerular filtrate is reabsorbed may be ascertained by determining its "clearance" simultaneously with the clearance of inulin or mannitol (p. 588), since the latter affords an index of the amount of material filtered through the glomerulus per unit of time.

Glucose. In normal men, under test conditions, the tubules can reabsorb 375 ± 80 mg. of glucose per minute (women, 305 ± 55 mg.). At a glomerular filtration rate of 125 cc./min., and a plasma glucose concentration of 280 mg./100 cc., 350 mg. of glucose would be entering the tubules each minute. The same quantity would be entering if the volume of glomerular filtrate were reduced to 75 cc./min. and the plasma glucose concentration were raised to 465 mg./100 cc. Under these circumstances, if tubular reabsorption was not impaired simultaneously, glucose would not necessarily escape in the urine despite the considerable elevation of blood sugar concentration (p. 58). The reabsorptive burden imposed upon the tubular cells is determined by the total quantity of substance presented to them per unit of time, i.e., concentration × volume, and not by its concentration alone. Reabsorption of glucose is largely an active process, apparently involving phosphorylation in the tubular epithelial cells. This is blocked by phlorizin (p. 61), with consequent glycosuria. Similar types of renal glycosuria occur clinically (p. 61 ff.).

WATER. Over 99 per cent of the enormous volume of water (about 180,000 cc. daily) that leaves the blood plasma in the glomeruli is normally returned to the blood in the tubules. Water is reabsorbed throughout the tubule, but by different mechanisms in different segments.

The fluid which enters the distal segment is approximately isotonic (sp. gr. about 1.010) with protein-free blood plasma (or glomerular filtrate), and its volume (about 16 cc./min.) is about 12.5 per cent of that of the glomerular filtrate. It follows, therefore, that 87.5 per cent of the water entering Bowman's capsule is reabsorbed isosmotically (i.e., with a corresponding amount of solute), in the proximal portion of the tubule. It is believed that this is a passive process, determined by the amount of solute (mainly sodium) reabsorbed. This phase of water reabsorption, representing over 80 per cent of the total, has therefore been designated "obligatory" reabsorption. Although the fluid at this point in the tubule is approximately isotonic with the original glomerular filtrate (i.e., same total osmolar concentration of solutes), it differs considerably from the latter in respect to the concentrations of individual components.

Inasmuch as the volume of the voided urine normally varies widely, depending upon existing circumstances, it is obvious that variable amounts of the water entering the distal segment are reabsorbed under different conditions. Since the specific gravity of the voided urine can vary widely below (hypotonicity) and above (hypertonicity) 1.010, it is clear that reabsorption of this fraction of the water is an active process, occurring independently of the amount of solute being reabsorbed simultaneously. This has been termed the "facultative" phase of water reabsorption, and it has been shown to be controlled by the antidiuretic hormone (ADH) of the posterior pituitary, which exerts its influence directly upon the epithelial cells in the distal segment of the tubule.

Secretion of the antidiuretic hormone is under the control of nerve fibers passing through the pituitary stalk from the supra-optic nuclei in the hypothalamus. Secretion of this principle may be interfered with either by resection of or injury to the posterior pituitary or by lesions in the hypothalamus involving either the supraoptic nuclei or the nerve pathways leading to the posterior pituitary. In the absence of the antidiuretic hormone and in the presence of some hormonal influence exerted by the anterior pituitary, probably the adrenocorticotrophic hormone, the capacity of the renal tubules to reabsorb water (facultative reabsorption) is reduced and polyuria results. This constitutes the condition known as diabetes insipidus, in which, when complete, about 12.5 per cent of the volume of glomerular filtrate may escape reabsorption, the urine volume rising to as much as 30,000 cc. daily, with a very low specific gravity (1.001–1.003). This condition may be corrected promptly by administration of adequate doses of posterior pituitary extract or of pitressin (p. 399).

Secretion of antidiuretic hormone is apparently regulated by the osmotic pressure (osmolar concentration) of the blood plasma. Decrease in plasma osmotic pressure of 0.1 per cent results in an increase of about 100 per cent in urine volume (i.e., water excretion). There is evidence that an increase in plasma osmotic pressure stimulates osmoreceptors in the hypothalamus, probably in the supraoptic nuclei, which have direct connections, via the pituitary stalk, with the secreting cells of the posterior pituitary. Consequently, minute variations in water content of the plasma are promptly corrected by appropriate changes in water excretion by the kidneys.

SODIUM, CHLORIDE, POTASSIUM. More than 99 per cent of the Na that leaves the plasma in the glomerular filtrate is reabsorbed in the tubules. It is believed that this is an active process, influenced significantly by hormones, particularly the "salt-active" hormones of the adrenal cortex, the most potent of which is aldosterone (p. 424). These stimulate Na reabsorption by the tubular epithelial cells, although the exact site and mechanism of their action are not known.

It is believed that about 80 to 85 per cent of the filtered Na is reabsorbed in the proximal segment, carrying with it a corresponding number of anions, mainly Cl, and water (obligatory water reabsorption). In the distal segment, Na reabsorption, in part at least, is involved in the regulation of acid-base balance, through the medium of the $Na^+ : H^+$ exchange mechanism (p. 330). This phase of Na excretion is therefore regulated to some extent by the pH of the blood plasma and the capacity of the distal tubular cells to acidify the urine, i.e., for H-ion formation (carbonic anhydrase) and ammonia formation (p. 331).

Potassium, too, undergoes extensive reabsorption (over 90 per cent) by the tubular epithelial cells. However, unlike Na, it is also excreted into the tubular lumen by cells in the distal segment. This active process, which competes with H ions in the $Na^+ : H^+$ exchange mechanism (p. 330), may be the phase of K excretion that is influenced by adrenocortical hormones, which enhance K excretion (p. 432).

UREA. About 40 per cent of the urea which leaves the blood in the glomerular filtrate is reabsorbed in the tubules under average normal conditions. This is a passive process, i.e., diffusion, the extent of urea reabsorption depending, therefore, on the amount of water being reabsorbed and on the concentration gradient between the intratubular fluid (i.e., modified glomerular filtrate), and the fluid in the tubular cells and kidney interstitial spaces.

It is to be expected, therefore, that the amount of urea reabsorbed (and excreted) would vary, within certain limits, with changes in urine volume. At low urine volumes, i.e., more water reabsorption, more urea is reabsorbed and less is excreted; at high urine volumes, i.e., less water reabsorption, more urea is excreted. Maximum urea reabsorption (about 70%) occurs at urine volumes of 0.35 cc. or less per minute. At a urine volume of 1 cc. per minute, about 60 per cent is reabsorbed, and at 2 cc. or more per minute 40 per cent is reabsorbed. If marked diuresis is induced by intravenous injection of an osmotically active substance, e.g., glucose or sucrose, the extent of urea reabsorption may fall considerably below 40 per cent, and values for urea clearance will approach those for inulin clearance, MISCELLANEOUS. Practically all of the normal components of the glomerular filtrate are reabsorbed in the tubules to extents varying from practically 100 per cent (e.g., glucose) or over 99 per cent (e.g., Na, water) to negligible amounts (e.g., creatinine). Many if not most of these are reabsorbed by active "tubular transport mechanisms," i.e., enzyme systems which effect their passage from the tubular lumen into the cells, and from the latter into the adjacent interstitial fluid. Reference will be made to a few of these which are of some clinical interest.

Phosphate is absorbed in the proximal segment. This process is influenced by the parathyroid hormone, which increases phosphate excretion (p. 227), apparently by decreasing its reabsorption. It has been suggested that this mechanism may be influenced also by vitamin D.

The bicarbonate of the glomerular fluid is almost completely reabsorbed ($<$ 25 mEq./liter) in the proximal segment, when present in normal concentrations. The remainder is reabsorbed in the distal segment, or is converted to CO_2 (which is then absorbed) as a result of the increasing acidity of the fluid in this portion of the tubule. If the plasma bicarbonate concentration is excessively high (alkalosis or alkali ingestion) some may escape reabsorption and be excreted in the urine.

About 90 per cent of the filtered uric acid is reabsorbed in the tubules. This, too, is an active process, interest in which has been stimulated by the demonstration of uricosuric effect of agents which block uric acid reabsorption, e.g., Benemid, carinamide. Such agents, particularly Benemid, appear to be valuable in the treatment of acute gout, lowering the plasma uric acid concentration as a result of their uricosuric action.

Under normal conditions, only small amounts of amino acids are excreted in the urine, the major fraction of what enters the glomerular filtrate being reabsorbed by active tubular transport mechanisms. Clinical conditions of excessive aminoaciduria, e.g., Fanconi syndrome, (p. 183), cystinuria (p. 184), occur in the presence of defects in these enzyme systems.

Tubular Excretion and Synthesis. The term excretion is applied to the active transport of substances by the tubule cells from the blood plasma to the lumen of the tubule. Synthesis refers to formation, in the tubule cells, of substances which are then passed into the lumen. Under physiological conditions, probably very few normal components of the plasma undergo significant active excretion by the renal tubular cells. Potassium is an important exception, and a portion of the urinary creatinine may be excreted by this route. However, a number of foreign substances that may be introduced into the body for therapeutic or diagnostic purposes are removed from the blood plasma extensively, and in certain instances even predominantly, by the tubular epithelium. Among these are penicillin, p-aminosalicylic acid, phenolsulfonphthalein (PSP), p-aminohippuric acid, and Diodrast. Recognition of this fact in the case of penicillin led to the use of transport-blocking agents for the purpose of maintaining effective plasma penicillin levels for longer periods by decreasing its excretion in the urine.

The use of the other substances named in the study of renal function is referred to elsewhere (p. 586).

There is evidence that in certain animals, compounds present in the urine are synthesized in the kidneys from precursors removed from the blood plasma, e.g., hippuric acid from glycine and benzoic acid. In man, such processes occur in the kidney to a very limited extent, if at all. However, the distal tubule cells do form two substances, hydrogen ions and ammonia, which, excreted into the lumen, play an extremely important part in the regulation of acid-base balance. This subject is discussed elsewhere in detail (p. 331).

Competition for Transport Mechanisms. There is evidence that several substances that are excreted by renal tubular cells are handled by a single mechanism, at least in one phase of their transport across the cell. The same is true of reabsorption of certain substances by these cells. The same mechanism, or one of its components, may be involved in reabsorption of certain compounds and excretion of others. Such substances may, under certain conditions, compete for these mechanisms, so that excretion or reabsorption of one or more is depressed by the presence of large amounts of another which utilizes the same mechanism.

Diodrast, p-aminohippurate, phenolsulfonphthalein, penicillin, and p-aminosalicylic acid, which are all actively excreted by tubule cells, exert mutually depressing effects on their urinary excretion when administered in sufficiently high dosage. Similar competition, in this instance for reabsorption, is exhibited between creatine, glycine, and other amino acids, between glucose and xylose, and between ascorbic acid and sodium chloride.

Reabsorption of ascorbic acid is depressed also by p-aminohippurate, which is excreted by the tubules. Carinamide and Benemid, although they are not actively excreted by the tubule, depress tubular excretion of diodrast, phenolsulfonphthalein, p-aminohippurate, penicillin, and p-aminosalicylic acid. They also decrease reabsorption of uric acid. Observations such as these have led to improvements in therapy (infections; gout).

CHARACTERISTICS OF NORMAL URINE

As indicated in the preceding discussion, the ultrafiltrate of the blood plasma, which leaves the circulation in the glomeruli, passes through the tubules and is subjected to various modifying influences, the nature and magnitude of which are determined, under normal conditions, by the requirements of the organism. The fluid which emerges from the collecting tubules is urine. It seems desirable to review certain important features of the composition of normal urine, an understanding of which may aid in the interpretation of changes that occur in disease.

Volume. The quantity of urine excreted daily by normal subjects varies widely and is determined chiefly by the fluid intake. Under ordinary dietary conditions it ranges from 1000 to 2000 cc. It is influenced somewhat by the protein and NaCl intake, the excreted urea and salt acting as diuretic agents. Excessive perspiration and strenuous exercise decrease the urine volume by prerenal deviation of water (skin, lungs) and, under abnormal conditions, the same is accomplished by vomiting, diarrhea, and edema. The amount excreted during the day (8 A.M. to 8 P.M.) is two to four times that excreted during the night (8 P.M. to 8 A.M.). The minimum volume required for excretion of a given quantity of solids is determined by the concentration capacity of the kidneys (p. 594). Under conditions of average adequate food intake, it is about 300 cc./sq.M. body surface (i.e., about 500 cc. for a 70-kg. man) (p. 296).

Specific Gravity. The specific gravity of the urine is directly proportional to the concentration of solute and, therefore, with any given total solid excretion, varies inversely with the volume under usual normal conditions. The grams of solute per liter may be calculated roughly by multiplying the last two figures of the specific gravity (to the third decimal) by Long's coefficient, 2.6. This is only an approximation, particularly since all substances do not exert the same effect on specific gravity. For example, the specific gravity of a liter of urine is raised 0.001 by 3.6 grams of urea, 1.47 grams of NaCl, and 3.8 grams of NaH_2PO_4 (also 2.7 grams of glucose and 3.9 grams of albumin).

Under ordinary dietary conditions, the specific gravity of the mixed twenty-four-hour urine usually varies between 1.014 and 1.026. However, it may be as low as 1.001 on a very high water intake or as high as 1.040 if fluid is restricted or excreted in excess by other routes (skin, bowel, etc.). In normal subjects it rarely exceeds 1.040, the maximum attainable osmotic concentration in man being about 1.4 osmolar.

Normal kidneys eliminate the required amount of solids regardless, within wide limits, of the amount of water available for their solution. When their function is impaired, the kidneys lose this ability to dilute and concentrate the urine, and, with increasing damage, the osmolar concentration and specific gravity of the urine approach values for protein-free blood plasma (glomerular filtrate). The maximum attainable specific gravity falls and the minimum rises, the urine specific gravity being relatively fixed within narrowing limits below and above 1.007. The importance of the urinary specific gravity arises out of these facts, determination of the concentrating ability of the kidneys constituting an important test of renal function.

Acidity. Urinary acidity may be expressed in two ways: (1) its H^+ ion concentration (true acidity); (2) its titratable acidity. These two factors (and also urinary ammonia) are responsive to the same influences, but are not necessarily related quantitatively. The pH may be regarded as an "intensity" factor and the titratable acidity as a "capacity" factor. Secretion of ammonia into the tubular urine decreases its H^+ concentration, playing an important role in the renal regulation of "acid-base" balance (p. 329).

pH OF NORMAL URINE. The extreme limits of urinary pH in health are 4.8 and 8.0, usually, under ordinary conditions, ranging between 5.0 and 7.0, averaging 6.0 in the mixed twenty-four-hour sample. The mechanism of acidification of the glomerular filtrate (pH 7.4) is discussed elsewhere (p. 330).

TITRATABLE ACIDITY. The titratable acidity of the urine is expressed as milliliters of N/10 alkali required to neutralize the twenty-four-hour output of urine. This usually varies normally from 200 to 500, averaging about 350. If phenolphthalein is used as the indicator, values below 250 usually indicate an actual alkalinity, since an end-point is attained at about pH 8.3. Because of the intimate relationship between urinary acidity and urinary ammonia (p. 331), the latter (usually 300 to 500 ml. of N/10 alkali) should be included with the former in studies of acid-base balance (i.e., titratable acidity plus ammonia).

The urinary acidity and pH are reflections chiefly of the ratio, NaH_2PO_4 : Na_2HPO_4, which is 1 : 4 at pH 7.4 (blood plasma; glomerular filtrate), about 50 : 1 at pH 4.8 and 9 : 1 at pH 6.0. This transformation of the dibasic to the monobasic salt effects considerable conservation of Na, which is returned to the plasma (p. 329). Acid urine contains virtually no bicarbonate (HCO_3^-). In alkaline urine, however, this ion is present and the pH may be determined largely by the ratio, H_2CO_3 : $NaHCO_3$. The urinary acidity is normally also contributed to, but to a minor extent, by organic acids (lactic, uric, hippuric, β-hydroxybutyric).

The acidity of the urine is normally influenced chiefly by the nature of the diet (i.e., alkaline or acid ash, p. 323). Ingestion of large amounts of protein (meats, bread, cereals), which yields acids in the course of metabolism (sulfuric, phosphoric), increases urinary acidity and ammonia. Most vegetables and fruits (orange, lemon, grape, apple, peach, pear) contain organic acids (e.g., citrate, oxalate) which form bicarbonate in the body and hence decrease urinary acidity. Certain fruits (plums, cranberries) contain benzoic acid and quinic acids, which are metabolized to and excreted as hippuric acid, increasing the urinary acidity and ammonia. These are increased also by starvation or dietary carbohydrate restriction, owing to increased catabolism of body protein and fat, with increased urinary excretion of sulfate, phosphate, and ketone acid (β-hydroxybutyric and acetoacetic).

Shortly after eating (within an hour) there is a decrease in urinary acidity and ammonia and [H^+] concentration. This "postprandial alkaline tide" has been attributed generally to the increased secretion of HCl by the stomach, temporarily increasing the plasma bicarbonate. It is said not to occur in subjects with achlorhydria. This explanation is not accepted by some. On standing, normal urine becomes alkaline and ammoniacal owing to the action of bacteria on urea (ammoniacal fermentation).

Nonprotein Nitrogenous Constituents. The term "nonprotein nitrogen" (NPN) is applied to nitrogen present in compounds not precipitable by the usual protein-precipitating agents. These substances, occurring in the blood and other body fluids and in the urine, consist chiefly of intermediary- or end-products of protein metabolism. They include urea, ammonia, amino acids, creatinine, creatine, uric acid, and a number of other compounds present in small amounts, not readily determined quantitatively, and referred to as "undetermined nitrogen" (peptides, hippuric acid, etc.).

The several components of this heterogeneous mixture have different origins and significance and are influenced by different factors; the amount of each excreted in the urine varies under different conditions of diet and metabolism.

The most important features of the biogenesis and metabolic significance of these substances are considered in the section on intermediary metabolism of proteins (p. 134 ff.) and nucleic acids (p. 199). Attention will be directed here mainly to their excretion in the urine of normal subjects.

Total Nonprotein Nitrogen. Normal adults on an adequate protein diet are in a state of nitrogen equilibrium, the quantity excreted daily being equivalent to that ingested. This is not the case during periods of tissue growth (children, pregnancy, convalescence) (positive nitrogen balance). Inasmuch as the amount of nitrogen excreted in the feces is relatively constant (p. 129) and that lost by other extrarenal channels is usually small and constant, the quantity in the urine may be regarded as reflecting the amount of protein ingested and the state of protein catabolism. It may vary from as little as 3 grams daily on a low protein intake (20 gm.) to as much as 24 grams or more on a very high protein diet (160 gm.); it ranges between 11 and 15 grams on usual protein intakes (70–80 gm.).

Table 40. Urinary Nitrogen Partition in Same Individual on High and Low Protein Diets
(After Folin, O.: J.A.M.A. *69*:1209, 1917)

	HIGH PROTEIN		LOW PROTEIN	
	(grams/24 hours)	(% of total N)	(grams/24 hours)	(% of total N)
Urine volume	1170 ml.		385 ml.	
Total N	16.80		3.60	
Urea N	14.70	87.5	2.20	61.7
Ammonia N	0.49	3.0	0.42	11.3
Uric Acid N	0.18	1.1	0.09	2.5
Creatinine N	0.58	3.6	0.60	17.2
Undetermined	0.85	4.8	0.29	7.3

Urea. Urea is the chief end-product of the catabolism of amino acids (p. 136) and is the substance in which is incorporated, for purposes of excretion, the bulk of the nitrogen provided to the organism in excess of its needs. Under usual dietary conditions it comprises about half of the total urine solids, and is therefore the most abundant urinary constituent (except water). Because of these facts, and because it is a metabolic end-product, virtually not utilizable, the quantity of urea excreted in the urine is the most significant index of the extent of protein catabolism and, consequently, is influenced more directly by the protein intake than are the other nitrogenous compounds.

The mechanism of excretion of urea is discussed elsewhere (p. 599). On a high protein diet, urea nitrogen (16–25 gm.) comprises 80 to 90 per cent of the urinary NPN. On very low protein intakes it may fall to 60 per cent or less of the total NPN, since variations in dietary protein are reflected almost exclusively in urinary urea and ammonia, the amounts of the other nitrogenous constituents remaining relatively constant (Table 40).

Ammonia. Unlike the other nonprotein nitrogenous constituents of the urine, preformed ammonia is not removed by the kidneys from the blood stream, in which ammonia is present in only minute amounts. It is formed in the renal tubular epithelium, chiefly (60%) from glutamine, the remainder from other amino acids (p. 136). It is passed largely into the tubular urine where, acting as a base, it combines with H^+ ions, forming NH_4, thus serving to lower the H^+ concentration and permitting further passage of H^+ ions into the lumen in exchange for Na^+ (p. 331). This mechanism is an important factor in the renal regulation of "acid-base" balance.

50 %. mu

Under usual dietary conditions, normal adults excrete about 0.3 to 1.2 grams (average 0.7 gm.) of ammonium N daily (20 to 70 mEq.), comprising 2.5 to 4.5 per cent of the total NPN. Inasmuch as the urea and ammonia of the urine are both derived ultimately from amino acids, an increase in ammonia is accompanied by a corresponding decrease in urea, at any given level of NPN excretion. The quantity of ammonia in the urine of normal subjects is determined chiefly by the requirement for acidification of the urine. The chief immediate stimulus to its formation in the tubular epithelial cells is apparently a decrease in the pH of the plasma and, consequently, in that of the urine in the tubules (p. 332).

The output of ammonia increases with increasing levels of dietary protein, owing probably to the acid-producing properties of protein (oxidation of S and P). It is increased also by ingestion of acids or acid-forming substances (NH_4Cl, NH_4NO_3) and in association with acidosis (except renal), and is decreased by ingestion of alkalis or base-forming foods and in alkalotic subjects. As much as 10 grams of ammonia may be excreted daily by individuals with severe diabetic acidosis. Administration of ammonium salts of mineral acids (viz., NH_4Cl, NH_4NO_3) is equivalent to administration of corresponding amounts of the free acids, since the ammonium is converted to urea in the body ($2NH_4Cl + CO_2 \rightarrow \text{Urea} + 2HCl + H_2O$). These ammonium salts therefore increase urinary acidity and can induce acidosis. If urine is allowed to stand without a preservative, its ammonia content increases markedly as a result of hydrolysis of urea by bacteria ("ammoniacal fermentation").

CREATINE AND CREATININE. The metabolic origin and significance of these substances are considered elsewhere (p. 150).

Creatine is not excreted in significant amounts in the urine of normal adult males. This is apparently due to the fact that it is completely reabsorbed from the glomerular filtrate in the renal tubules at normal plasma creatine levels (< 0.6 mg./100 cc.). It is present in the urine (increased plasma level) of prepuberal children (4.2 mg./kg. daily) and certain normal women, periodically or constantly, and occasionally during pregnancy and the early puerperium.

Under normal conditions, creatine formed in or otherwise provided (food) to the organism over and above the requirements or capacity for storage in the muscles (as phosphocreatine, p. 11) is either transformed to creatinine or excreted as such. The creatine tolerance test is a measure of the capacity for utilization of creatine. Normal adult men excrete in the urine (in twenty-four hours) not more than 20 per cent and women not more than 30 per cent of a test dose (1.32 or 2.64 gm.) of creatine hydrate. Urinary creatine is increased in conditions accompanied by muscle wasting, muscle dystrophies, and thyrotoxicosis.

Creatine nine (creatine anhydride) is formed from creatine in quite constant amounts daily (men, 1.0–1.8 gm.; women, 0.7–1.5 gm.), corresponding to about 2 per cent of the body creatine, practically all of which is eliminated in the urine. The excretion of creatinine is therefore constant from day to day under quite varied conditions, being relatively uninfluenced by diet or urine volume, and being determined chiefly by the muscle mass (creatine content of body). Because of this fact, the daily excretion of creatinine is determined commonly in balance studies as a check on the accuracy of twenty-four-hour urine collections. Endogenous creatinine is excreted in men by glomerular filtration and active tubular excretion, the value for creatinine clearance (about 145) exceeding that for inulin (p. 582).

The term "creatinine coefficient" is applied to the number of milligrams of creatinine (plus creatine, if any) nitrogen excreted daily per kilogram of body weight. Normal values are 20 to 26 for men and 14 to 22 for women. The corresponding values in terms of nitrogen are 7.5 to 10 for men and 5 to 8 for women. The creatinine coefficient may be regarded as an index of the amount of muscle tissue, and is therefore more directly proportional to the ideal than to the actual weight.

AMINO ACIDS. Amino acids are excreted in the urine in both free and combined form. The latter are present in peptide linkage, as in compounds such as hippuric acid (glycine). The free amino nitrogen ranges normally from 0.1 to 0.15 gram daily, comprising 0.5 to 1.0 per cent of the total nitrogen. The total amino nitrogen output is 0.5 to 1.0 gram daily (2–6% of the total nitrogen). The organism conserves amino acids efficiently, the amount excreted by normal subjects varying but slightly with wide fluctuation in protein intake and even when amino acids are administered parenterally. Severe liver damage results in an increase in urinary amino acids and a corresponding decrease in urea (impaired deamination and urea formation).

URIC ACID. Uric acid is the chief end-product of the metabolism of purines. Its metabolic origin is considered elsewhere (p. 199). Normal values for urate clearance range from 11 to 15 ml. per minute; consequently, about 85 to 90 per cent of the urate filtered through the glomeruli is reabsorbed in the tubules.

In urine of average pH (6.0 or higher) uric acid is present largely as the soluble sodium and potassium urates, whereas in highly acid urine the relatively insoluble free acid may predominate and may precipitate from solution.

The amount of uric acid in the urine of normal subjects depends on the quantity of nucleoprotein ingested (exogenous) and that formed from tissue nucleic acids and nucleotides (endogenous). On purine-free diets, the urinary uric acid is of solely endogenous origin and is rather constant for the individual, usually 0.1 to 0.5 gram daily. This may be increased somewhat by strenuous exercise and by a high protein or high caloric intake (stimulating endogenous metabolism).

On a high purine diet (meat, liver, kidney, sweetbreads, leguminous vegetables, etc.) the uric acid output may be as much as 2 grams daily. In adults on the usual mixed diet the average daily excretion is about 0.7 gram, comprising 1 to 2 per cent of the total nitrogen. The urine of newborn infants contains relatively large amounts of uric acid (0.2 gm./100 cc.), comprising about 7 to 8 per cent of the total nitrogen. Crystals of free uric acid and ammonium urate may be present and urate "infarcts" of the kidneys are not uncommon. A sharp drop to normal adult levels occurs after seven to ten days. A considerable increase follows administration of cinchophen and salicylates. Caffeine and other methylpurines (in tea, coffee, cocoa) cause little or no increase, being either transformed largely to urea or undergoing more complete destruction in the body.

Urinary excretion of uric acid, reflecting nucleoprotein catabolism, is increased by 11-oxygenated adrenocortical hormones and by ACTH. It has been suggested that determination of the extent of rise in the urinary uric acid : creatinine ratio after administration of ACTH may be useful as a test of the reserve functional capacity of the adrenal cortex (p. 441), but this has not proved to be of much value.

Allantoin. Allantoin is formed from uric acid in the liver by the action of the enzyme uricase (p. 199). In man and anthropoid apes, the livers of which exhibit little or no uricase activity, allantoin is excreted in very small amounts (5–25 mg. daily). In other mammals it is the chief end-product of purine metabolism, accounting for 90 per cent or more of the excretion of purine metabolites. It was formerly believed that the metabolism of purines in the Dalmatian dog resembled that in man and apes (differing from that of other mammals) in having uric acid as its chief metabolic end-product. Present evidence indicates that the urinary excretion of relatively large amounts of uric acid is due to its incomplete reabsorption by the renal tubular epithelium and not to a difference from other breeds of dogs in capacity for forming allantoin from uric acid.

Oxalic Acid. Small amounts of oxalate (10–25 mg.) are excreted daily in the urine of normal subjects. Its origin is not well understood. Calcium oxalate, which precipitates from neutral or alkaline urine in the presence of abnormally high oxalate excretion, is an important constituent of urinary calculi. Under normal conditions, the main source of urinary oxalate is probably dietary, it or certain metabolic precursors being present in many common foodstuffs (tomatoes, rhubarb, cabbage, spinach, asparagus, apples, grapes, etc.). Oxalate may also be one of the end-products of the metabolism of ascorbic acid and may also be formed, perhaps, in the intermediary metabolism of carbohydrate, presumably not significantly under normal conditions.

Glucuronic Acid. Glucuronic acid occurs in normal urine conjugated with a wide variety of compounds in two types of linkage, (1) ester and (2) glycosidic. The carboxyl groups of such compounds as phenylacetic acid and benzoic acid form ester linkages, whereas the hydroxyl groups of such substances as aliphatic or aromatic alcohols (chloral, camphor, phenol, menthol, indole, skatole, morphine, pregnanediol, estriol, etc.), form glycosidic linkages. This conjugation apparently takes place in the liver, which is probably also the site of formation of glucuronic acid, the metabolic derivation of which is not clear.

The normal daily excretion of glucuronic acid is about 0.3 to 1.0 gram. This may be increased by administration of the substances indicated above, as well as acetylsalicylic acid, sulfonamides (except sulfanilamide), turpentine, antipyrine, and phenolphthalein. When present in relatively high concentration, the ester types reduce the usual copper reagents (e.g., Benedict's), probably owing to concomitant hydrolysis, and may therefore interfere with the interpretation of urinary tests for glucose. The glycoside types do not reduce these reagents unless previously hydrolyzed.

Hippuric Acid (Benzoylglycine). Hippuric acid is formed by the conjugation, in peptide linkage, of benzoic acid and glycine. In man, this occurs largely if not solely in the liver; in certain species (e.g., dog), it occurs also in the kidney.

Benzoic acid (or benzoate) is present, as such, in many vegetables and fruits (e.g., cranberries, plums) and addition of sodium benzoate, as a preservative, to certain prepared foodstuffs is legally permissible. Quinic acid, another constituent of vegetables and fruits, is converted to benzoic acid in the body; the latter may arise also from intestinal bacterial action on aromatic compounds, viz., tyrosine and phenylalanine.

Glycine is normally invariably present in amounts adequate to convert all of the benzoic acid formed to hippuric acid. The latter, formed in the liver cells, enters the systemic circulation and is excreted in the urine in amounts ranging from 0.1 to 1.0 gm. (usually 0.5–0.7 gm.) daily, depending largely on the dietary intake.

Citric Acid. Citrate is excreted in the urine in amounts of 0.2 to 1.2 gm. daily. It is increased by administration of estrogen and decreased by androgen. Urinary citrate varies during the menstrual cycle, increasing during the first half (increasing elaboration of estrogen), persisting at a high level during the luteal phase (synergistic action of estrogen and progesterone), and falling in the premenstrual phase (drop in estrogen). The significance of these changes is not clear. Administration of alkalis or alkaline ash diets increases, and acidification decreases urinary citrate.

Citric acid is formed in large amounts in the course of oxidative metabolism of carbohydrate. Whether or not this is the main source of urine citrate is not known. Citrate is present in the bones, apparently as a superficial deposit on the crystal lattice of apatite, which is the chief mineral complex in bone (p. 229). Citrate forms soluble and undissociable complexes with calcium, and these two ions exhibit certain interrelationships in their urinary excretion. Administration of parathyroid hormone or of very large doses of vitamin D, producing hypercalcemia, increases urine calcium and citrate (mobilization from skeleton?). Injection of citrate increases urinary excretion of calcium, perhaps by increasing its solution from bone.

Other Organic Acids. Lactic acid, the end-product of glycolysis in muscle, passes into the blood stream, undergoing ultimate reconversion to glycogen or glucose in the liver (p. 11). About 50 to 200 mg. are excreted daily in the urine under ordinary conditions of activity. Much more may appear during periods of prolonged, strenuous muscular exercise, or anoxia.

Minute amounts (up to 50 mg. daily) of fatty acids may be excreted in the urine. These are mainly short-chain acids (formic, acetic, butyric), which arise chiefly during intermediary metabolism and as a result of intestinal bacterial action.

Certain aromatic hydroxyacids may appear in the urine of normal subjects in small amounts. The most important are p-hydroxyphenylacetic and p-hydroxyphenylpropionic acids, which are formed in the intermediary metabolism and intestinal putrefaction of tyrosine. Other aromatic hydroxyacids (p-hydroxyphenylpyruvic, p-hydroxyphenyllactic, dihydroxyphenylacetic acids) may be excreted in abnormal states (tyrosinosis, vitamin C-deficient infants, certain cases of mental deficiency, alkaptonuria).

Ketone Bodies. The substances designated "ketone bodies" include acetone, acetoacetic acid, and β-hydroxybutyric acid. These are formed in the liver in the course of metabolism of fatty acids (p. 90), and are conveyed in the blood stream to the tissues for further oxidation. Under normal conditions, on the usual mixed diet, less than 125 mg. are excreted daily in the urine. Larger amounts may be eliminated on high-fat diets, during starvation or severe carbohydrate restriction, and during normal pregnancy. Marked increases are encountered in a variety of abnormal states associated with excessive ketogenesis (p. 94), e.g., diabetes mellitus (p. 75).

Sulfur-Containing Compounds. Sulfur appears in the urine as (1) so-called neutral sulfur and (2) sulfate, the latter in (*a*) inorganic and (*b*) organic (ester; ethereal sulfate) combination. The bulk of the urinary sulfur is derived from the metabolism of the amino acids, methionine, cystine, and cysteine, the amount excreted by normal subjects therefore varying with the protein intake.

The total sulfur excretion usually ranges from 0.7 to 1.5 grams daily, averaging about 1.0 gram. The urinary N : S ratio is about 15 : 1. Neutral sulfur comprises about 5 to 15 per cent of the total (0.04–0.15 gm.), and sulfate-S 85 to 95 per cent. About 5 to 15 per cent of the latter is organic (ethereal) sulfate (0.06–0.12 gm.), the remainder inorganic (0.6–1.0 gm.).

Neutral sulfur includes such substances as cystine, methionine, methylmercaptan, taurine and its derivatives (taurocholic acid), thiocyanate, thiosulfate, and urochrome.

Ethereal (organic) sulfate consists largely of phenolic sulfates, formed in the liver from aromatic compounds arising from intestinal bacterial action on tyrosine and tryptophan (indole, skatole, cresol, phenol) or from benzene or other phenolic compounds entering the organism (as under conditions of industrial or accidental exposure). Certain hormones, including estrone and androsterone, are excreted largely as sulfates and fall into this category (pp. 402, 413). Indole, which arises almost entirely from intestinal putrefaction, is converted to indoxyl in the liver, is conjugated with sulfate, and appears in the urine as potassium (and sodium) indoxyl sulfate (indican). This is a rough index of the extent of intestinal putrefaction (< 20 mg. daily normally).

Further details are considered in the section on sulfur metabolism (p. 263).

Phosphate. The metabolism of phosphorus is considered elsewhere (p. 224 ff.).

Phosphorus is excreted (urine and feces) largely as inorganic phosphate, and small extent in organic form ($< 4\%$ of total). Inasmuch as the phospha derived chiefly from the metabolism (oxidation) of phosphorus-contain ganic foodstuffs (and tissue components), such as phosphoproteins, nucle teins, nucleotides, and phospholipids, the quantity excreted depends in l measure on the nature of the diet. It is therefore extremely variable.

The proportion of the total phosphorus excretion eliminated in the u depends under normal conditions on dietary and intra-intestinal factors. the usual mixed diet the daily urinary phosphorus excretion is 0.7 to 1.5 gr averaging about 1.1 grams, almost entirely in the form of inorganic phosph comprising about 60 per cent of the total excretion (feces 40%). If intes absorption of phosphate is diminished, as by high intake of calcium or magnes (also aluminum, beryllium, iron), which tend to form relatively insol phosphates, unusually high intestinal alkalinity, and low vitamin D intake children), the amount and proportion of phosphorus eliminated in the u decreases (increases in feces). Conversely, on a low calcium, moderately phosphorus intake, about 75 per cent of the total excretion is urinary. parathyroid hormone increases renal excretion of phosphate by diminishin reabsorption in the tubules (p. 227). The "renal threshold" for phosp excretion is about 2 mg. P/100 cc. plasma, excretion falling to a minimu lower concentrations.

The phosphate ion appears in the urine in two forms, viz., acid phosph $H_2PO_4^-$, and basic phosphate, $HPO_4^=$. The ratio, $BH_2PO_4 : B_2HPO_4$, v with and largely determines the pH of the urine, since acid and basic phosph constitute the major buffer system in that fluid. Relatively insoluble phosph tend to form in alkaline urine, and are often important components of ur calculi. The turbidity which develops in urine on standing (ammoniacal fer tation) is contributed to largely by precipitation of phosphates, inclu $MgNH_4PO_4$ ("triple phosphate"), insoluble at alkaline reactions.

Chloride. In normal subjects on an average diet (8–15 gm. NaCl intake), quantity of chloride in the urine is second only to urea, among the solid stituents, approximating the intake (5–9 gm. chloride ion; 110 to 225 ml The normal "renal threshold" for chloride is about 340 mg./100 cc. plas in normal subjects, as the plasma chloride concentration descends to this le urinary excretion of chloride decreases (renal tubular reabsorption). Th influenced by the level of adrenal cortical function.

If the chloride intake is changed suddenly, some time may be required for reestablishment of equilibrium between intake and output. For example, sudden addition of a large amount of salt is usually followed by elimination o excess within forty-eight hours. However, if the previous intake has been sually low, elimination of the increment may be delayed several days.

In conditions in which elimination of chloride through other chann increased, e.g., excessive perspiration, diarrhea, vomiting, the urine chlo decreases correspondingly.

Sodium; Potassium. The kidney is the chief regulator of excretion, o

tly of the body equilibrium of sodium and potassium, the chief cations of
cellular and intracellular fluids, respectively. Operation of these excretory
anisms therefore plays an important role in the maintenance of water and
l-base" equilibrium (pp. 316, 329). The daily urinary output of these
ents by normal adults approximates their intake. Under usual dietary condi-
, normal adults excrete in the urine about 3 to 5 gm. (130–215 mEq.)
um and 2 to 4 gm. (50–100 mEq.) potassium daily (Na : K ratio about
). During periods of fasting or inadequate protein intake, excessive tissue
in catabolism, with liberation of intracellular fluid components, results in
crease in urinary potassium and a change in the Na : K ratio.

renal cortical hormones, under ordinary circumstances, stimulate reab-
tion of sodium and may depress that of potassium by the renal tubular epithe-
cells (p. 432). Potassium enters the urine from the plasma by glomerular
ation and also by active tubular secretion, the latter mechanism apparently
peting with that for urine acidification. Details of this process are considered
where (p. 330). The amount of potassium in the urine is therefore influenced
he requirement for urine acidification, increasing when this requirement is
(e.g., alkaline ash diet; alkalosis) and decreasing when it is high (highly acid
diet; acidosis).

bnormal loss, through extrarenal channels, of sodium (excessive sweating,
rhea, etc.) or of potassium (diarrhea, vomiting) results in decrease in the
ary excretion of these elements.

cium; Magnesium. Under usual dietary conditions in normal subjects,
ary excretion accounts for about 15 to 30 per cent of the total calcium and
ut 20 to 50 per cent of the total magnesium elimination, the remainder being
reted in the feces. At low or moderate levels of calcium intake (0.1–0.5 gm.
y), about 30 to 50 per cent is eliminated in the urine, whereas at high levels
ntake (1.0 gm.) about 10 to 25 per cent is so eliminated. The dependence of
urinary excretion of these elements on their absorption from the intestine,
the factors involved (vitamin D, phosphate, etc.), are considered elsewhere
. 231, 232).

The "renal threshold" for excretion of calcium lies between 6.5 and 8.0 mg.
100 cc. plasma, little being eliminated at lower plasma calcium concentra-
s. The influence of the parathyroid hormone in increasing urinary calcium is
ondary to its action in increasing mobilization of calcium from the bones into
blood stream (p. 231). The effect of vitamin D in increasing urine calcium
consequence of its enhancement of absorption of calcium from the intestine
225).

rbohydrates (p. 19). Normal urine may contain reducing substances (< 1.5
ns/24 hours), up to 40 per cent of which are fermentable; concentrations
r 0.25 per cent (Benedict's test) should be regarded as probably abnormal. A
tion of this is glucose, the remainder being derived chiefly from the diet
actose from lactose of milk; pentose from fruits; caramelized sugar and
trins; proteins; products of intestinal bacterial action).

lucose may appear in the urine in small amounts during normal pregnancy, particularly in the later months. In certain normal individuals, glucose may be excreted after ingestion of large amounts of sucrose, glucose, or, at times, starch (alimentary glucosuria). Lactose is excreted frequently during lactation. Pentose (L-arabinose) may appear in the urine of normal subjects after they have eaten large quantities of foods rich in pentose, e.g., cherries, grapes, plums, prunes (alimentary pentosuria). There is a chronic, familial type of pentosuria, in which L-ketoxylose is excreted independently of the nature of the diet. Galactose and fructose rarely appear in the urine of entirely normal subjects except after administration of supertolerance doses of these sugars.

Miscellaneous. The urine of normal subjects may contain protein in amounts too small to be detected except by very sensitive procedures. The normal urinary protein does not exceed 75 mg. daily, and consists of serum albumin, which passes through the glomerular filter, enzymes (e.g., pepsin, trypsin, lipase, amylase), and nucleoprotein and "mucin" derived from the lower urinary tract.

The twenty-four-hour urine may contain minute quantities of iron (< 0.3 mg.), arsenic (< 0.5 mg.), copper (< 0.1 mg.), zinc (< 0.6 mg.), iodine (< 0.07 mg.), and trace amounts of cobalt, nickel, fluorine, silicon, and lead.

A number of the water-soluble vitamins and their metabolites are excreted in the urine, usually in proportion to their intake. The quantities eliminated in the urine under controlled conditions have been employed as a basis for clinical evaluation of the state of vitamin nutrition, and are considered in the discussion of the individual vitamins (q.v.).

Certain hormones or their metabolites are excreted in the urine, e.g., gonadotrophins (p. 383 ff.), estrogens (p. 402), androgens (p. 413), pregnanediol (p. 409), adrenocortical hormones (p. 430). Determination of the quantities eliminated is employed in the clinical evaluation of the state of functional activity of the respective endocrine glands (q.v.).

Normal urine contains only minute amounts of lipids. Rarely, fat may appear in the urine after ingestion of unusually large amounts of fat, e.g., cod-liver oil ("alimentary lipuria").

CLINICAL STUDY OF RENAL FUNCTION

Present understanding of renal physiology permits rather precise evaluation of certain individual phases of kidney function. Measurements can be made of the rate of glomerular filtration, the renal blood flow, and the maximum capacity of the tubules for excretion and for reabsorption. Certain of the test procedures required for precise quantitative determination of these factors do not lend themselves as yet to routine general clinical use, although they are so used in many clinics devoted to the study of patients with renal and hypertensive diseases. However, procedures are available for general use which provide satisfactorily quantitative information on essential aspects of renal functional activity. The methods of study employed most widely at the present time fall into three categories: (*a*) tests based on the "clearance" concept; (*b*) studies of excretion of certain dyes, preferentially excreted by the kidneys; (*c*) studies of the concentrating capacity of the kidneys.

CLEARANCE TESTS

Urea clearance is defined as the volume of blood which one minute's excretion of urine suffices to clear of urea. Inasmuch as all of the blood flowing through the kidneys is only partially cleared of urea (and other substances), a more exact definition would be "the number of cubic centimeters of blood which contain the amount of urea removed per minute by renal excretion." The clearance also represents the minimum volume of blood required to furnish the amount of substance excreted in the urine in one minute. This concept of renal clearance has been expanded to include a number of substances other than urea and has contributed largely to our present understanding of renal function in health and disease.

If U indicates the concentration (mg. per 100 cc.) of substance (e.g., urea) in urine, and V the volume (in cc.) of urine formed per minute, $U \times V$ equals the quantity of substance excreted per minute. If B indicates the concentration (mg. per 100 cc.) of substance in the blood, UV/B indicates the virtual volume of blood "cleared" of substance per minute, i.e., the "clearance." It is essential that plasma be used instead of whole blood for clearance determinations if the substance investigated is not distributed uniformly between plasma and corpuscles (e.g., inulin, Diodrast, phenol red).

Theoretically, a substance may be excreted by (*a*) glomerular filtration alone, (*b*) filtration plus tubular excretion or (*c*) filtration plus tubular reabsorption. If a substance is completely filtered at the glomerulus and is subsequently completely reabsorbed by the tubules, its clearance will be zero (e.g., glucose). As the degree of tubular reabsorption diminishes, the substance appears in the urine and its clearance increases (e.g., urea), until, if there is no reabsorption (e.g., inulin), the clearance will be equivalent to the rate of glomerular filtration. If a substance, in addition to being filtered through the glomeruli, is also excreted by the tubular epithelium (e.g., phenol red, Diodrast), its clearance will exceed the rate of glomerular filtration by an amount equal to the extent of tubular clearance. Inasmuch as the kidneys cannot excrete more of a substance per unit of time than is brought to them in the blood, the upper limit of renal clearance is determined by the renal blood flow. For example, if a substance undergoes glomerular filtration and tubular excretion, and if all that is contained in the blood passing through the kidneys is removed and is concurrently transferred to the urine, its clearance will be complete (e.g., Diodrast clearance); i.e., it will be equivalent to the volume of plasma flowing through the kidneys per minute. These facts constitute the basis for quantitative determination of various aspects of renal function.

Glomerular Filtration. Inulin is a polysaccharide which is not metabolized in the body and, following its intravenous injection, is excreted quantitatively by the kidneys within a short time. It is excreted entirely by glomerular filtration and undergoes no reabsorption in the tubules. Inasmuch as inulin, being freely filtrable at the glomerulus, exists in the blood plasma and glomerular filtrate in identical concentration, and since the quantity of inulin excreted per minute in the bladder urine is equal to the amount entering the glomerular filtrate per minute, it follows that the inulin clearance (UV/P) represents the volume glomerular filtrate formed per minute. Employing this procedure, it has be found that the average rate of glomerular filtration is 125 cc. per minute (ab 70 cc. per sq. meter of body surface). Similar figures are obtained with ot polysaccharides, viz., mannitol, sorbitol, and dulcitol. Lower values (by 2 45%) may be obtained in elderly (70 to 85 years), apparently normal subjec

Determination of the rate of glomerular filtration (inulin clearance) has seve important physiologic applications: (*a*) the glomerular filtration rate minus t rate of urine flow (bladder) equals the quantity of water reabsorbed in the tubu

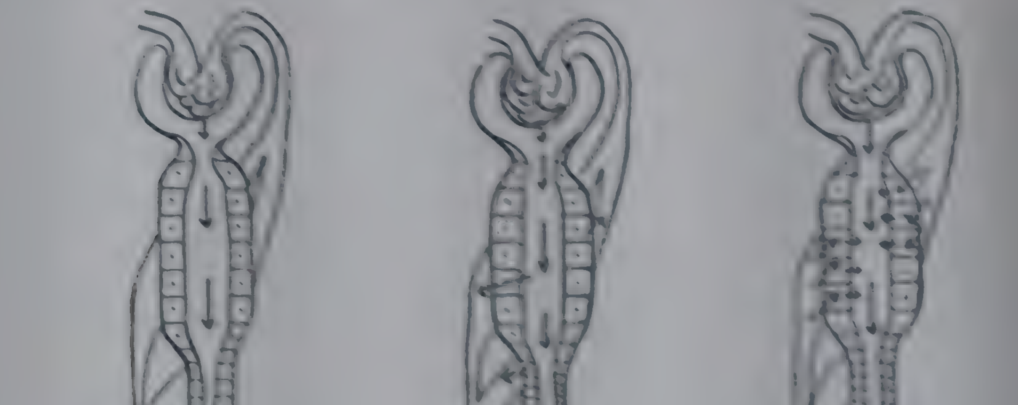

Fig. 54. Characteristic clearance values. Scheme to illustrate mechanisms of excret Inulin is excreted by glomerular filtration alone, with no tubular reabsorption. Urea is excre by glomerular filtration and is partially reabsorbed by the tubule. Diodrast is excreted by b glomerular filtration and tubular excretion.

per minute; (*b*) the inulin clearance minus the clearance of another substan (X) divided by the inulin clearance, equals the proportion of substance X rea sorbed in the tubules. For example: Inulin Clearance (125) minus Urea Cleara (75), divided by Inulin Clearance (125), equals 0.4, indicating that 40 per cent the urea of the glomerular filtrate is reabsorbed during its passage through t tubules; (*c*) when a substance has a clearance value higher than that of inu it is excreted partly or entirely by the tubules (p. 568). Such data have be found to be useful in studying the mode of action of diuretic agents and abn malities in the excretion of such substances as sodium, glucose and uric ac

Glomerular filtration is influenced by: (*a*) renal blood flow; (*b*) glomeru blood pressure and plasma colloid osmotic pressure (p. 562); (*c*) capsular pr sure (p. 563); (*d*) the filtration surface area, i.e., the functioning glomeru surface area. Abnormalities in the rate of glomerular filtration (i.e., inulin itol clearance) may occur, therefore, in a variety of clinical disorders in which factors are altered.

Diminished Renal Blood Flow. Under normal conditions an enormous ime of blood (p. 586) passes through the glomeruli each minute, about 18 to er cent of the plasma (except protein) filtering into Bowman's capsule. her factors remain unchanged, a significant decrease in blood flow through kidneys may result in a decrease in the volume of glomerular filtrate. The rtant causes of diminished renal blood flow are outlined elsewhere (p. 586). necessary to indicate here, however, that this does not invariably result in ased filtration because of the simultaneous occurrence, in certain conditions, her changes which tend to increase the filtration pressure.

rious fall in systemic arterial blood pressure due to left ventricular (e.g., nary occlusion) or peripheral circulatory (e.g., shock, hemorrhage) failure companied by reduction in renal blood flow and glomerular filtration. How- , the decrease in filtration is not proportional to that in blood flow, and in arterial hypotension it may not be altered significantly, due to simultaneous triction of the efferent glomerular arterioles, the filtration pressure being by maintained or increased. A similar phenomenon occurs in congestive t failure, in which the reduction in renal blood flow exceeds that in filtration. is probably due to renal arteriolar constriction, affecting the efferent rioles predominantly, in response to the increased venous pressure, with equent increase in the filtration fraction (p. 588).

essential hypertension, constriction or sclerosis of the glomerular arterioles, ominantly the afferent, results in diminution in renal blood flow and glomeru- iltration pressure and, consequently, in the volume of glomerular filtrate.

Glomerular Damage. Significant decrease in the surface area of the nerular filter should result in reduction in the volume of filtrate, unless the l blood flow and/or filtration pressure were increased correspondingly, a ation which is virtually never encountered clinically. In fact, the renal d flow is diminished in practically all types of diffuse glomerular damage of ficant degree because of the obstacle imposed to the flow of blood, almost all hich must pass through the glomeruli (p. 562).

lomerular filtration may be decreased, therefore, in diseases of the kidneys racterized by diffuse glomerular damage. These include glomerulonephritis, hrosclerosis, intercapillary glomerulosclerosis, renal amyloidosis, and ad- ced bilateral chronic pyelonephritis, etc.

. Increased Capsular Pressure. Resistance to filtration is increased in presence of obstruction to the passage of fluid through the uriniferous tubule ower urinary passages. This raises the pressure in Bowman's capsule and eases the effective filtration pressure (p. 563). Conditions in which this or may operate include: blockage of renal tubules by desquamated cells, , and other precipitated material, e.g., hemoglobin, Bence-Jones protein, acid, calcium salts; obstruction in any portion of the lower urinary tracts by uli, inflammation, stricture, neoplasm, etc.

4. Altered Plasma Colloid Osmotic Pressure. Significant elevation of plasma C.O.P. is rarely encountered, since increase in the plasma protein concentration, when it occurs, is virtually invariably in the globulin fraction (p. 152), which exerts relatively little influence on osmotic pressure. On the other hand, decrease in plasma C.O.P. occurs commonly, due to the frequent occurrence of hypoalbuminemia (p. 156). This should result in an increase in effective glomerular filtration pressure (p. 563) and increased filtration, if other factors remain unchanged. Whether this actually occurs is uncertain, but an increased glomerular filtration rate has been observed in children with chronic nephrosis (severe hypoproteinemia) which may be attributable to this cause, although increased renal blood flow has also been incriminated.

Determination of glomerular filtration (inulin or mannitol clearance) is o obvious value in investigating the pathological physiology of renal function i disease. Inasmuch as all of the normal constituents of the urine probably leave th blood stream exclusively via the glomerular filtrate, with the exception of potassium (p. 330) and a relatively small fraction of the creatinine, reduction i glomerular filtration is usually the most important primary cause of abnorma retention in the body fluids of urinary waste products.

In interpreting deviations from normal values for inulin or mannitol clearance i.e., glomerular filtration rate (Table 41), certain points should be kept in mind Determinations should be made under carefully controlled and standardize conditions, including performance in the morning after an overnight fast (diurna variation), adequate antecedent diet (dietary influences), avoidance of emotiona disturbance (hormonal influences), and bed rest (exercise influence). Moreove there may be some question of the validity of measurements of glomerular filtration in certain kidney diseases, particularly in the presence of severe acute tubula damage, e.g., mercury or carbon tetrachloride poisoning, severe pyelonephritis In such conditions, a portion of the filtered inulin or mannitol may diffuse out o the lumen across the damaged, nonfunctioning tubular cells, with the consequen production of spuriously low filtration values. However, this occurs rarely, eve in advanced stages of the types of renal disease commonly encountered.

The usefulness of inulin and mannitol clearance tests as routine clinical diagnostic procedures is limited by the necessity of injecting the test substanc intravenously and by certain other technical difficulties. In normal subjects, an often in disease states, under average conditions of urine flow, values for ure clearance usually parallel those for inulin clearance, and the latter may be calculated from the former according to the formula: Urea Clearance/0.6 = Inuli Clearance. This proportionality arises out of the fact that all of the urinary urea i removed from the blood by glomerular filtration and, at urine volumes of 2 cc or more per minute, about 60 per cent is excreted in the urine (40% reabsorbed (p. 602). This substitution of urea clearance for inulin clearance is not valid a low urine volumes. Nevertheless, except under unusual circumstances, determination of urea clearance is entirely satisfactory in the clinical study of rena function and for purposes of diagnosis and prognosis (p. 603).

Renal Blood Flow. If a substance is completely removed from the bloo

FACTORS	MEN	WOMEN	GLOMERULONEPHRITIS			CHRONIC PYELO-NEPHRITIS	BENIGN NEPHRO-SCLEROSIS
			ACUTE	Chronic			
				Nephrotic	Terminal		
Glomerular filtration rate (GFR) Inulin clearance Mannitol clearance (cc./min.)	130 ± 20	115 ± 15	15–100 (50)	40–80 (55)	0–40 (10)	50–90 (75)	50–150 (100)
Renal plasma flow (RPF) PAH clearance Diodrast clearance (cc./min.)	700 ± 135	600 ± 100	60–800 (400)	400–700 (500)	20–500 (100)	300–700 (425)	450–700 (500)
Renal blood flow (cc./min.)	1275 ± 245	1090 ± 180					
Filtration fraction (%)	18–20	18–20	10–25 (15)	10–20 (15)	10–30 (25)	15–20 (17)	10–30 (22)
Maximum tubular excretion (Tm) PAH Tm (mg./min.)	75 ± 13	70 ± 10	10–80 (50)	30–40 (35)	10–60 (30)	10–50 (35)	50–100 (75)
Diodrast Tm (mg. I/min.)	55 ± 15	50 ± 15					
Maximum tubular reabsorption (Tm) Glucose Tm (mg./min.)	375 ± 80	300 ± 55					
Plasma flow per unit excretory mass RPF/Diodrast Tm (cc./mg.)	9 ± 1.5	8 ± 1.5					
RPF/PAH Tm (cc./mg.)	9 ± 1.5	8 ± 1.5	6–18 (8)	10–20 (12)	6–18 (10)		7–10 (8)
Plasma flow per unit reabsorption mass RPF/Glucose Tm (cc./mg.)	2 ± 0.4	2 ± 0.5					
Filtration per unit functioning nephrons Inulin clearance/PAH Tm (cc./mg.)	1.7 ± 0.4	1.5 ± 0.3	0.8–2.2 (1.2)	0.9–1.8 (1.2)	1.0–4.5 (1.5)		1.0–2.2 (1.5)

plasma during its passage through the kidney, the "clearance" of that subs
(i.e., volume of plasma "cleared" per minute; $\frac{UV}{P}$ (p. 581) would equa
renal blood plasma flow. Knowledge of the hematocrit value would pe
calculation of the renal blood flow. This has been found to be true of Dio
and p-aminohippurate, over 90 per cent of the plasma content of these subst
being removed during a single passage through the kidneys ("extraction ra
at relatively low plasma levels. Consequently, at least in normal subjects u
standardized conditions, values for Diodrast or p-aminohippurate are app
mately equivalent to the "effective" renal plasma flow, i.e., the flow thr
active renal excretory tissue. These have been found to be about 700 ±
cc. for men, and 600 ± 100 cc. for women, per 1.73 square meters of b
surface. Corresponding values for renal blood flow are about 1275 ± 24
and 1090 ± 150 cc., respectively. Lower values are obtained in elderly
dividuals.

Renal blood flow exhibits a diurnal variation, decreases during exercise, a
influenced by emotional states. These determinations must therefore be m
under strictly standardized conditions. p-Aminohippurate (PAH) has la
replaced Diodrast for estimation of renal blood flow, because it possesses
following advantages over the latter substance: (*a*) it does not penetrate
erythrocytes; (*b*) it is less extensively bound to plasma proteins; (*c*) its quan
tive determination is a simpler procedure. Values for PAH clearance in ex
mental animals correspond closely to those obtained by direct measureme
renal blood flow, amounting to about 30 per cent of the cardiac output and a
25 per cent of the total blood volume. Stated in other terms, all of the b
passes through the kidneys every four minutes, a fact which reflects the
importance of these organs in maintaining a constant extracellular fluid c
position.

Abnormal decrease in "effective" renal blood flow (PAH clearance)
result from:

1. Decrease in cardiac output or arterial blood pressure, e.g., conge
heart failure, coronary artery occlusion, shock syndrome.

2. Organic disease of the renal vascular system, e.g., renal arterioscler
arteriolosclerosis (nephrosclerosis), intercapillary glomerulosclerosis, glomer
nephritis, periarteritis nodosa.

3. Increased local resistance to the flow of blood, resulting from vasocons
tion of the afferent and/or efferent glomerular arterioles, e.g., early ess
hypertension, systemic arterial hypotension (p. 522).

4. Decrease in the mass of functioning kidney tissue, e.g., renal hypopl
polycystic disease, pyelonephritis, tuberculosis, malignancy.

An absolute increase in renal blood flow may occur after administrati
pyrogenic agents, e.g., typhoid vaccine, due to renal arteriolar dilatation. It
be increased temporarily by a high meat diet. Following unilateral nephrect
the blood flow through the remaining kidney may increase more than 70 per
in a short time.

e significance of diminished renal blood flow in contributing to reduction in erular filtration is referred to elsewhere (p. 583). The fact must be borne in that measurement of renal blood flow by the clearance procedure is valid when renal extraction of PAH or Diodrast is almost complete, i.e., when 90 per cent is removed during a single passage through the kidneys. Inasmuch er 80 per cent of the normal excretion of these substances is accomplished rect tubular cell action and less than 20 per cent by glomerular filtration, e impairment of tubular excretory function may result in reduction in PAH odrast clearance in the absence of corresponding diminution in renal blood . This will be reflected in decrease in the PAH extraction ratio, which can etermined by simultaneous measurements of PAH in the systemic arterial d and in the renal vein blood obtained by catheterization. By this means, extraction ratios have been observed in subjects with severe kidney damage o glomerulonephritis or nephrosclerosis. In such cases, the renal blood flow be calculated by substituting the arterial-venous plasma difference in PAH Diodrast) concentration for "P" in the clearance formula, i.e. $\frac{U_{PAH} \cdot V}{P(\text{art-ven})_{PAH}}$. ever, PAH extraction is usually normal in early stages of these conditions, also in congestive heart failure.

ximum Tubular Excretory Capacity. The process of renal tubular etion is limited by the mass of functioning tubular tissue available for the fer of a substance from the blood to the urine. Consequently the measure t of the maximal rate of excretion of a substance excreted by this mechanism ts the "tubular excretory mass" of the kidneys. This value, designated Tm, lculated from simultaneous determinations of inulin and Diodrast or p-amino urate clearances at high plasma Diodrast or PAH levels. It is expressed as igrams of Diodrast iodine or PAH eliminated per minute by tubular excretion, total excretion minus the amount excreted by glomerular filtration. Normal es for Diodrast and PAH Tm are indicated in Table 41 (p. 585).

has been suggested that the Tm may be calculated indirectly as follows:

$$\text{Tm} = \frac{\text{Sp. Gr.} - 3.4}{4.8}\sqrt{\text{UC}},$$

e Sp. Gr. represents the second and third decimal place figures of the mum urinary nonprotein specific gravity obtained by the concentration test 95) (viz., 1.028 = 28.0) and UC is the urea clearance in terms of per cent erage normal.

e value of this determination lies in the fact that, at effective plasma PAH iodrast concentrations and with adequate but not necessarily normal renal l flow, the Tm is independent of glomerular activity and reflects the amount nctioning renal tubular tissue. For example, if one kidney were removed, ould be diminished by 50 per cent; if a portion of the excretory tissue were yed (either tubular destruction or obliteration of circulation), Tm would duced in proportion to the extent of destruction. If the glomeruli were entirely obliterated without impairing the circulation to the tubules, Tm would be unaltered.

The ratio of renal plasma flow to maximum tubular excretory capacity, i.e., of PAH or Diodrast clearance to PAH or Diodrast Tm, is an expression of the plasma flow per unit of functioning excretory renal tissue. Normal values are indicated in Table 41 (p. 585). Increased values may be obtained in the presence of fever (active hyperemia), and decreased values (relative ischemia) in the presence of intrarenal vasoconstriction, e.g., in shock, essential hypertension, peripheral circulatory failure, and in severe anemia.

Percentage Tubular Reabsorption. If another substance as freely filtrable through the glomeruli as inulin has a clearance value lower than the latter, it has undergone reabsorption in the tubules. The extent of this reabsorption may be calculated as follows:

$$\text{Reabsorption} = \frac{\text{Inulin Clearance (e.g., 125) minus Clearance of X}}{\text{Inulin Clearance (125)}}.$$

By means of this calculation it is evident that about 40–50 per cent of the urea present in the glomerular filtrate is normally reabsorbed in the tubules under average conditions of urine flow (p. 602). $\left(\frac{125 - 75}{125} = 0.4 \text{ or } 40\%.\right)$

Filtration Fraction. The ratio of plasma inulin clearance (i.e., volume of glomerular filtrate) to plasma PAH or diodrast clearance (i.e., renal plasma flow) represents the fraction of plasma filtered through the glomeruli. Under normal conditions this is about 0.18 (i.e., $\frac{125}{700}$), indicating that approximately 18 per cent of the water of the plasma flowing through the kidneys is filtered through the glomeruli into the lumen of Bowman's capsule.

Experimental studies have shown that increase or decrease in the filtration fraction is usually due to increase or decrease, respectively, in the tone of the efferent as compared to that of the afferent glomerular arteriole, with consequent increase or decrease, respectively, in intraglomerular blood pressure. Renal hyperemia produced by a pyrogen is probably due to predominantly efferent arteriolar dilatation, since the renal blood flow increases and the filtration fraction decreases. The tone of the efferent arteriole is increased by administration of epinephrine and in orthostatic and psychogenic vasoconstriction, with consequent decrease in renal blood flow and increase in the filtration fraction. In early essential hypertension, as well as in hypertension induced by administration of renin or angiotonin (experimental), the renal blood flow (diodrast clearance) is usually decreased, glomerular filtration (inulin clearance) is often normal, and the filtration fraction is frequently increased; this combination of circumstances can be produced practically only by predominantly efferent arteriolar constriction. Because of the increased filtration fraction, glomerular filtration and urea clearance may be maintained within normal limits until late in the course of essential hypertension (p. 620); under such circumstances, the normal urea clearance is not an expression of absolute integrity of renal function, but is usually an indication of renal vasoconstriction. An increase of filtration fraction from 0.2 to 0.3 is adequate to maintain urea clearance at 100 per cent of normal at a time when renal blood flow has fallen from a normal level of 1000 cc. per minute to an ischemic level of 600 cc. per minute.

Maximum Tubular Reabsorption Capacity. This may be studied in a manner similar to that employed for determination of excretory Tm, except that in this case a substance must be employed that is reabsorbed extensively by the tubular epithelial cells, rather than excreted by them. Glucose is used for this purpose. When the plasma glucose concentration is maintained at a sufficiently high level, the amount of glucose entering the tubule will exceed the reabsorptive capacity and some will escape in the urine. Simultaneous determination of inulin clearance and mean blood glucose concentration permits calculation of the amount of glucose entering the tubule per minute, since all of it enters in the glomerular filtrate. Subtraction of the quantity escaping in the urine per minute gives the amount that has been reabsorbed.

Normal values for glucose Tm are indicated in Table 41 (p. 585). The significance of abnormalities in this factor is much the same as in the case of the excretory Tm (PAH or Diodrast), applying in this instance to tubular reabsorptive mass. The plasma flow per unit of functioning tubular reabsorptive mass may be calculated by determining the ratio of renal flow (PAH or Diodrast clearance) to the glucose Tm (Table 41).

Glomerular function in terms of active nephrons may be expressed by the ratio of glomerular filtration rate (inulin clearance) to maximum tubular excretory capacity (PAH Tm). Normal values are indicated in Table 41. Increased values reflect hyperfiltration and decreased values hypofiltration in residual nephrons.

Other Clearance Procedures. At physiological levels of plasma creatinine (i.e., endogenous), values for creatinine clearance correspond rather closely to those for inulin clearance in normal subjects. However, when the concentration in the plasma is raised by intravenous injection of creatinine, or in renal disease, the clearance values exceed those for inulin (rising to about 175 cc.), indicating partial tubular excretion of this substance under these conditions. Consequently, the creatinine clearance is not an invariably reliable index of the glomerular filtration rate.

Phenolsulfonphthalein has an average clearance value of 400 cc., indicating that it is eliminated mainly (about 70%) by tubular excretion. This fact serves to explain occasional discrepancies between values for PSP excretion and urea clearance in disease states (p. 614), urea being excreted entirely by glomerular filtration, a portion being reabsorbed subsequently.

Renal clearances of other substances are determined occasionally in studying the pathological physiology of certain conditions, e.g., uric acid in gout and eclampsia, sodium in congestive heart failure. However, they have little place in the clinical evaluation of renal functional efficiency. Studies of urea clearance are employed most widely for this purpose; this subject is discussed in detail elsewhere (p. 602).

ELIMINATION OF WATER

Comparison of Fluid Intake and Fluid Output (Fig. 22, p. 297). Th quantity of water eliminated by the kidneys in twenty-four hours depends up two factors, namely, the amount of water supplied to the body and the amou lost through extrarenal channels or otherwise rendered unavailable for excreti by the kidneys.

The organism receives water from three main sources. These are consider elsewhere in detail and need be outlined here only briefly.

(*a*) Ingested liquids.

(*b*) Ingested solids. This source of supply is too frequently overlooked. Th water content of many solid foods is extremely high, and, in some instances, higher than that of certain foods commonly considered to be liquid. For examp tomatoes contain a greater percentage of water than does milk. In any accura study of water balance the water content of so-called "solid foods" must taken into consideration.

(*c*) Water of combustion, or metabolic water. 100 gm. of dry starch metab ized in the body form 55.5 gm. of water, and a similar quantity of glucose, gm. of water. 100 gm. of fat metabolized in the body form approximately gm. of water. This source of supply of water to the organism usually represe but a small proportion of the total supply, however, and does not introduc serious error in the calculation of the water intake.

The kidneys ordinarily excrete more than one half of the water eliminat from the body. The basal requirement of water for urine formation is depend chiefly upon the intake (or catabolism) of protein and NaCl, inasmuch as u and NaCl comprise the bulk of the solid constituents of the urine. Under norm conditions the remainder is lost largely through the vaporization of water in t respiratory passages and by the skin. The average quantity lost daily in this way 850 cc. In the presence of active perspiration or fever this amount is increa and that eliminated by the kidneys is correspondingly diminished. The amou of water excreted by normal kidneys may be greatly decreased in the presence conditions which cause depletion of the water reserves or abnormal retention water in the body, as indicated below.

These extrarenal factors must be taken into consideration when studying re function on the basis of the quantitative relationship between water supply a urine volume. Ordinarily the latter is simply compared with the fluid intal The total water supply, in individuals on an average diet, exceeds the fluid int by about 700 cc.; in the absence of the pathological factors mentioned ab the water loss exceeds the urine volume by approximately the same amou Therefore, under such circumstances, the consideration of fluid intake as t water supply and urine volume as total water elimination involves an erro approximately only 5 per cent. Interpretations based upon such observati should be made, however, with full realization of the inherent possibility error, particularly in the presence of extrarenal factors which may influence water balance.

Oliguria; Anuria. Decrease in urine volume (oliguria) occurs in a variety of clinical disorders and, if marked, may of itself produce renal insufficiency in the absence of intrinsic kidney damage, or may aggravate renal functional impairment due to kidney disease. Oliguria (or anuria) may be due to prerenal, renal, or postrenal (lower urinary tract) causes.

DEHYDRATION (p. 312). Excessive loss of water and electrolytes by extrarenal routes (prerenal deviation of water) results in a corresponding decrease in urine volume unless compensated by an increased intake. Oliguria of this type occurs in a number of conditions accompanied by prolonged vomiting or diarrhea, excessive sweating, profuse expectoration (lung abscess, bronchiectasis), profuse inflammatory effusions, external intestinal or bile fistulas, etc. In the absence of significant kidney damage, the specific gravity of the small volume of urine is high. If the quantity of water available for solution of the required amount of urinary solids is inadequate for this purpose, some will be retained in the body, as reflected in an increase in blood urea N. A similar situation may develop as a consequence of prolonged diuresis, e.g., in uncontrolled diabetes mellitus, with depletion of available body water reserves (p. 312).

ARTERIAL HYPOTENSION. Maintenance of glomerular filtration requires an adequate effective filtration pressure; this is dependent primarily on the glomerular blood pressure, which, in turn, depends on the general arterial pressure (renal artery). Filtration cannot be maintained at mean arterial pressures much below 70 mm. Hg, and anuria develops. This may occur in peripheral circulatory collapse (shock), as seen frequently after severe trauma, and extensive burns postoperatively, following acute hemorrhage, in acute coronary occlusion, overwhelming infections, and as a consequence of severe dehydration.

Conditions in this category are among the most common causes of oliguria and renal functional insufficiency, often with blood urea N retention. The kidneys may be entirely normal initially. The fall in blood pressure, whether due to blood loss, decreased plasma volume, peripheral vasodilatation, or decreased cardiac output, is followed by intrarenal arteriolar constriction, the efferent glomerular arterioles being affected disproportionately, with consequent rise in glomerular blood pressure and increase in the filtration fraction (p. 588). This mechanism tends to maintain glomerular filtration at lower levels of arterial pressure than would be possible otherwise. However, this is accomplished at the expense of renal blood flow, which, diminished originally as a result of the hypotension, or of the underlying phenomena, is further reduced by the increasing intrarenal vasoconstriction. Under these circumstances, glomerular filtration ultimately becomes inadequate and an unduly large fraction of the filtrate that is formed is reabsorbed in the tubules.

Moreover, tubular damage, even to the extent of severe necrosis, may develop as a consequence of the renal ischemia (anoxia) resulting from the reduced renal blood flow. Under these conditions, e.g., "lower nephron nephrosis," oliguria and anuria are contributed to by the unselective reabsorption of the glomerular filtrate across the now nonfunctioning tubular epithelial lining. Superimposition of this type of kidney damage upon the original circulatory disturbance is of serious prognostic significance, the gravity of this condition increasing with increasing duration of the hypotensive state (i.e., shock).

Edema. In edema due to any cause, prerenal deviation of water results in decrease in urine volume. The several factors involved in abnormal retention of water in the body in various clinical disorders are reviewed elsewhere (p. 314). Intrinsic kidney disease may or may not be present. However, disturbance of tubular function, i.e., increased reabsorption of Na and water, may occur in the absence of significant morphological kidney damage, e.g., in congestive heart failure and hepatic cirrhosis (p. 316).

Kidney Disease. Oliguria may result from diffuse glomerular damage (e.g., glomerulonephritis) of sufficient degree to reduce glomerular filtration to very low levels. It occurs also even in the presence of adequate glomerular filtration, if there is extensive tubular degeneration (necrotizing nephrosis), as in poisoning by mercury, arsenicals, carbon tetrachloride, and sulfonamides, and in the shock syndrome and so-called "lower nephron nephrosis" (severe trauma, burns, etc.). In such conditions, the tubular functions of selective reabsorption are lost in the damaged areas, and back-diffusion of the glomerular filtrate occurs as a result of loss of the physiological barrier (tubule cells) between the fluid in the lumen of the tubules and the concentrated blood in the peritubular capillaries. In severe cases the continuity of the tubule may actually be disrupted at some points, permitting direct passage of the glomerular filtrate into the peritubular interstitial spaces.

Urinary Obstruction. This may be intrarenal or extrarenal. Flow of the fluid through the tubules may be impeded as a result of occlusion of the lumen by casts or other precipitated material (crystals, hemoglobin derivatives, Bence-Jones protein), desquamated epithelial debris, or compression by edematous renal interstitial tissue. Back-pressure may be increased also as a result of bilateral lower urinary tract obstruction (e.g., urinary calculi; ureteral stricture, tumor, or compression; prostatic enlargement; bladder tumors; urethral stricture, etc.). Under these circumstances the capsular pressure (p. 562) is increased, filtration is reduced, and an unduly large fraction of the smaller filtrate undergoes reabsorption as a result of its slower passage through the tubule.

Water Function Test (Dilution Test). This test possesses the advantage of putting a strain upon the water excretory function of the kidneys. It may be performed as follows:

(*a*) The fasting individual, after emptying the bladder, ingests 1200 cc. of water in one-half hour.

(*b*) The bladder is emptied at hourly intervals for four hours.

In the normal subject approximately 1200 cc. are eliminated within four hours, the larger part being excreted in the first two hours. The specific gravity of one of the hourly specimens should fall to at least 1.003. If renal function is impaired the quantity eliminated in four hours may be quite small and the specific gravity often 1.010 or higher, although lower figures are obtained not infrequently. Identical results may be obtained if any of the extrarenal factors mentioned above are operative. The differentiation between renal and extrarenal causes of defective water excretion must depend upon the results of other tests of renal function. The value of the dilution test lies in its clinical availability; it rarely adds any significant information to that obtained by other methods.

The ability of the kidney to eliminate water may be impaired in all types of renal disease, acute or chronic. This impairment may appear to be most pronounced in those cases associated with oliguria and edema. However, inability on the part of the kidneys to excrete water is not the fundamental factor in the pathogenesis of edema.

The edema of acute or chronic nephritis and the nephrotic syndrome is probably due largely to prerenal deviation of fluid into the tissues, caused by increased capillary permeability, decrease in the colloid osmotic pressure of the blood plasma (decreased plasma albumin concentration), or increase in capillary blood pressure (congestive heart failure) (p. 315). The marked degree of oliguria which is an outstanding feature of such cases is largely secondary to the extrarenal factors mentioned above.

The existence of polyuria in chronic nephritis may mask a definitely impaired ability to eliminate water. Polyuria in such cases must be regarded as a compensatory mechanism whereby the kidney, its ability to excrete solids in their normal concentration being impaired, must dilute these substances in order to secure their adequate elimination. As in the case of other organs, such as the heart, this compensation is effected at the expense of the functional reserve capacity. Therefore, when such a test as the dilution or water function test is performed and 1200 cc. of water are ingested within one-half hour, only a small fraction may be eliminated in the succeeding four hours. If such a patient can excrete only 400 cc. in the four hours, a markedly deficient water excretion, he might still maintain a polyuria of 2400 cc. in twenty-four hours.

ELIMINATION OF SOLIDS—URINE SPECIFIC GRAVITY

Considered in a broad sense, the chief excretory function of the kidney is to eliminate solid substances in solution in water. Many of these substances exist in the urine in much higher concentration than in the blood, the ratio of the average concentration in the urine to its concentration in the blood during the same period (concentration ratio) varying greatly for each of the urinary constituents. Thus, in the normal performance of its excretory functions the kidney must concentrate the eliminated substances, the necessary degree of concentration at any moment depending upon the relative quantities of solids and water present at that moment in the blood passing through the glomerular capillaries. One of the most important characteristics of the healthy kidney is its ability to eliminate the required quantity of solids regardless, within wide limits, of the amount of water available for their solution. In other words, the normal kidney exhibits a remarkable flexibility in its concentrating ability. Consequently, the concentration of solid constituents of normal urine, as evidenced by the specific gravity, varies considerably during the day in accordance with the ingestion of fluids and solid food and with the metabolic activity of the tissues. If large amounts of fluids are ingested the urine is of large volume and low specific gravity; if little water is of this type of kidney damage upon the original circulatory disturbance is of serious prognostic significance, the gravity of this condition increasing with increasing duration of the hypotensive state (i.e., shock).

EDEMA. In edema due to any cause, prerenal deviation of water results in decrease in urine volume. The several factors involved in abnormal retention of water in the body in various clinical disorders are reviewed elsewhere (p. 314). Intrinsic kidney disease may or may not be present. However, disturbance of tubular function, i.e., increased reabsorption of Na and water, may occur in the absence of significant morphological kidney damage, e.g., in congestive heart failure and hepatic cirrhosis (p. 316).

KIDNEY DISEASE. Oliguria may result from diffuse glomerular damage (e.g., glomerulonephritis) of sufficient degree to reduce glomerular filtration to very low levels. It occurs also even in the presence of adequate glomerular filtration, if there is extensive tubular degeneration (necrotizing nephrosis), as in poisoning by mercury, arsenicals, carbon tetrachloride, and sulfonamides, and in the shock syndrome and so-called "lower nephron nephrosis" (severe trauma, burns, etc.). In such conditions, the tubular functions of selective reabsorption are lost in the damaged areas, and back-diffusion of the glomerular filtrate occurs as a result of loss of the physiological barrier (tubule cells) between the fluid in the lumen of the tubules and the concentrated blood in the peritubular capillaries. In severe cases the continuity of the tubule may actually be disrupted at some points, permitting direct passage of the glomerular filtrate into the peritubular interstitial spaces.

URINARY OBSTRUCTION. This may be intrarenal or extrarenal. Flow of the fluid through the tubules may be impeded as a result of occlusion of the lumen by casts or other precipitated material (crystals, hemoglobin derivatives, Bence-Jones protein), desquamated epithelial debris, or compression by edematous renal interstitial tissue. Back-pressure may be increased also as a result of bilateral lower urinary tract obstruction (e.g., urinary calculi; ureteral stricture, tumor, or compression; prostatic enlargement; bladder tumors; urethral stricture, etc.). Under these circumstances the capsular pressure (p. 562) is increased, filtration is reduced, and an unduly large fraction of the smaller filtrate undergoes reabsorption as a result of its slower passage through the tubule.

Water Function Test (Dilution Test). This test possesses the advantage of putting a strain upon the water excretory function of the kidneys. It may be performed as follows:

(*a*) The fasting individual, after emptying the bladder, ingests 1200 cc. of water in one-half hour.

(*b*) The bladder is emptied at hourly intervals for four hours.

In the normal subject approximately 1200 cc. are eliminated within four hours, the larger part being excreted in the first two hours. The specific gravity of one of the hourly specimens should fall to at least 1.003. If renal function is impaired the quantity eliminated in four hours may be quite small and the specific gravity often 1.010 or higher, although lower figures are obtained not infrequently. Identical results may be obtained if any of the extrarenal factors mentioned above are operative. The differentiation between renal and extrarenal causes of defective

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being evacuated, the test period urine may be of low specific gravity, simulating diminished concentrating ability. Obviously, under such conditions a low maximum specific gravity is not necessarily indicative of renal disease.

This simple test is one of the most valuable tests of renal function. A great deal of information may be obtained by the use of the concentration test in conjunction with the estimation of the nonprotein nitrogenous constituents of the blood. The former indicates the presence or absence of renal functional impairment; the latter indicates whether this is in the compensated or uncompensated stage.

The relative values of the concentration tests and the determination of blood urea clearance are discussed elsewhere (p. 614 ff.). The latter gives more definite information regarding the actual degree of renal functional impairment, but the former is quite as sensitive usually in detecting the presence of such impairment. From a practical standpoint, if normal results are obtained by the concentration test, renal function may generally be regarded as normal and determination of the blood urea clearance is usually superfluous. Exceptions to this generalization are indicated elsewhere.

Significance of Hyposthenuria. Observations in experimental animals indicate that the fluid entering the distal segment of the uriniferous tubule is approximately isotonic with protein-free plasma (sp. gr. about 1.010), although the concentrations of certain components have been changed. Inasmuch as the capacity for concentrating this fluid must therefore reside mainly in functions of the distal tubular cells, diminished concentrating ability (hyposthenuria) is a reflection chiefly of impaired function of the distal portion of the tubule. However, this is not necessarily dependent upon demonstrable morphological changes in these cells. Although the mechanisms underlying hyposthenuria in certain conditions are not entirely clear, it may usually be attributed to one or more of the following factors:

1. Tubular Epithelial Damage. Tubular degeneration (nephrosis) may result from: (*a*) nephrotoxic agents, such as mercury, carbon tetrachloride, uranium, and sulfonamides; (*b*) severe alkalosis; (*c*) the shock syndrome, due to a variety of causes ("lower nephron nephrosis"); (*d*) secondary to glomerular damage, due to impairment of tubular blood supply (p. 562), as in glomerulonephritis and advanced nephrosclerosis.

If the damage is very severe, e.g., in necrotizing nephroses, anuria may occur initially (back-diffusion). In less severe cases there may be a low output of urine of low specific gravity. During recovery from severe damage, polyuria may occur (low urine specific gravity) and, subsequently, hyposthenuria may persist for several weeks or months. This is apparently a reflection of functional inadequacy of the newly regenerated tubular epithelial cells.

In so-called "lipoid" nephrosis, the urine volume is characteristically low (edema) and the specific gravity high. This indication of satisfactory tubular function despite visible morphological damage is supported by observations of normal ammonia formation and urine acidification in this condition.

2. Decreased Renal Blood Flow. A considerable amount of osmotic work must be performed (i.e., energy produced) by the tubule cells in forming urine

of a specific gravity higher than that of protein-free blood plasma. This metabolic activity is reflected in the relatively high oxygen consumption of kidney tissue. It would be expected, therefore, that these cells should be particularly vulnerable to anoxia, and that their functional activity should be impaired by reduction in renal blood flow. Hyposthenuria occurs under these circumstances, and also in severe chronic anemias. The conditions in which renal blood flow is diminished are outlined elsewhere (p. 586). In many of these, initially, there may be no visible morphological damage, but regressive changes develop if the circulatory impairment (or anemia) is marked and prolonged.

3. DECREASED NUMBER OF FUNCTIONING NEPHRONS. Removal of a large portion of kidney tissue in experimental animals results in polyuria and hyposthenuria. Although the mechanism is not known exactly, there is evidence that the number of glomeruli is reduced proportionately more than the renal blood flow. Consequently, each glomerulus is perfused with an abnormally large volume of blood, and the minute volume of glomerular filtrate may be increased to such an extent that the reabsorption capacity of the tubules is exceeded (relative tubular insufficiency). This results in hyposthenuria. A similar situation may occur at times in certain cases of chronic pyelonephritis and other destructive kidney lesions (e.g., tuberculosis, malignancy), and occasionally in glomerulonephritis and nephrosclerosis.

As indicated above, hyposthenuria occurs in almost all diseases of the kidneys and in certain types of renal functional impairment due to extrarenal causes. Investigation of the maximal concentrating capacity of the kidneys (concentration test) is therefore a valuable approach to the study of kidney function. However, it has certain limitations.

Misleading low specific gravity findings may be obtained during periods of elimination of edema fluid, e.g., improvement in congestive heart failure, chronic nephrosis, hepatic cirrhosis, etc. This is, of course, not a reflection of impaired concentrating ability, but a consequence of the increased water excretion during the test period.

Variable results are often obtained in essential hypertension (benign nephrosclerosis). In many cases, hyposthenuria cannot be demonstrated in the early stages of the disease, and indeed, in some instances, for prolonged periods during the evolution of the renal lesion (nephrosclerosis). Clearance studies during this period reveal reduction in effective renal plasma flow, glomerular filtration, and maximal tubular excretory capacity, reflecting significant renal functional abnormalities that may not be revealed by urine concentration tests in this condition. Hyposthenuria occurs almost invariably in the later states of nephrosclerosis.

An essentially normal concentrating capacity may be exhibited in conditions in which glomerular damage is not accompanied by significant tubular damage or functional impairment (uncomplicated glomerular insufficiency). This occurs at times in early stages of acute glomerulonephritis and also in prerenal deviation of water (p. 604). In such cases, the reduction in glomerular filtration may result in marked oliguria and elevation of blood urea N, despite the fact that the specific gravity of the inadequate volume of urine may be quite high. As a rule, hypothenuria occurs later in the course of these conditions, if they persist or increase severity, because of the superimposition of tubular damage as a result of prolong curtailment of blood supply (p. 616).

Whereas decrease in maximal concentrating capacity, with certain excepti indicated above, denotes impairment of kindey function, the extent of such d crease (i.e., of hyposthenuria) does not always reflect accurately the degree renal functional inadequacy. In chronic glomerulonephritis, for example, hyp thenuria may progress rather rapidly to isosthenuria, i.e., fixation of speci gravity at about 1.010, long before the urea clearance has fallen to its ultima terminal levels. During recovery from acute glomerulonephritis, hyposthenu often persists for some time (weeks or months) after glomerular filtration a renal blood flow have returned to normal. This may reflect functional inadequa of the newly regenerated tubular epithelial cells (p. 617).

ELIMINATION OF NONPROTEIN NITROGENOUS SUBSTANCI

The methods of study of the ability of the kidneys to eliminate the nonprot nitrogenous end-products of protein metabolism may be classified as follow

(1) Urinary Studies.
(2) Simultaneous Urinary and Blood Studies.
(3) Blood Studies.

Urinary Studies

Studies of urinary nonprotein nitrogenous elements from the standpoint the investigation of renal function have been largely directed toward urea a creatinine. These studies have been essentially of two varieties: those design to determine the ability of the kidneys to eliminate a known quantity of pr formed urea or creatinine (balance studies), and those designed to determine t ability of the kidneys to concentrate urea (urea concentration tests). This ty of study is not employed extensively at the present time, having been larg replaced by the more informative clearance studies.

Simultaneous Study of Blood and Urine

Urea Excretion Ratio. Addis and Watanabe (1916) suggested that the ra between the rate of urea excretion and the blood urea concentration shou constitute an accurate index of the functional efficiency of the kidneys. Th factor, termed the urea excretion ratio, is expressed as follows:

$$\text{Ratio, } \frac{U}{B} = \frac{\text{Urea in 1 hour's urine}}{\text{Urea in 100 cc. blood}} = 50 \text{ (average normal).}$$

Addis believed this ratio to be independent of the urine volume, but, as w later demonstrated, this is true only when the rate of urine excretion exceeds cc. per minute (augmentation limit). If a urine volume in excess of this quant is ensured under the conditions of the test, this ratio is a useful index of ren functional efficiency.

t was later pointed out by Austin, Stillman, Van Slyke and their associates that urea excretion ratio of Addis was identical in principle, and, with minor thematical corrections, identical in fact with their "maximum blood urea arance" values. Since the determination of blood urea clearance possesses a der field of clinical application, the procedure suggested by Addis will not be cussed in further detail. It is essentially the same as that to be considered ow.

ood Urea Clearance. As a result of investigations of the nature described ve, it became evident that the most exact information regarding the urea reting ability of the kidney requires comparisons of the blood urea concentra- n and the urea excretion in the urine. It had been shown that with fairly large ne volumes the rate of elimination of urea is directly proportional to the od urea content. Austin, Stillman and Van Slyke demonstrated that this direct ationship holds only when the urine volume exceeds a certain limit, 2 cc. per nute (adults), which they designated the "augmentation limit." With ne volumes below this figure, the rate of urea elimination was found to , and was found to be, on the average, proportional to the square root the urine volume. Further investigations indicated that perhaps the most isfactory medium of expression of the relationship between the blood urea ncentration and the urinary urea excretion was by means of the "blood urea arance," which is expressed as the number of cubic centimeters of blood com- tely cleared of urea by renal excretion. The blood of course is not completely ared of urea in its passage through the glomerulus. About 10 per cent of the od urea is so removed. Therefore, if, under conditions of maximum blood a clearance, about 750 cc. of blood plasma pass through the kidney per minute, e amount of urea removed would be equivalent to that contained in 75 cc. of sma. A more exact definition of blood urea clearance would be "the number of bic centimeters of plasma which contain the amount of urea removed per nute by renal excretion."

It was shown that, in normal adults, when the excretion of urine proceeds at rate of 2 cc. or more per minute, a certain volume of blood plasma will be ed of urea each minute. This volume of plasma, termed the "maximum arance" (Cm), normally ranges from 64 to 99 cc., the mean value for an adult average size (surface area of 1.73 square meters) being 75 cc. of blood per nute. Expressed as a formula,

$$Cm = \frac{UV}{P},$$

ere U designates the milligrams of urea in 100 cc. of urine, P the milligrams of a in 100 cc. of blood plasma and V the urine volume in cubic centimeters r minute.

Similarly, when the urine output is below the augmentation limit (less than c. per minute), a certain volume of blood plasma will be freed of urea each nute. This volume of plasma is termed the "standard clearance" (Cs) and varies normally from 41 to 65 cc., the mean value for an adult (surface area 1.73 square meters) being 54 cc. per minute. Expressed as a formula,

$$Cs = \frac{U}{P}\sqrt{V}.$$

Because of the observation that the blood urea clearance, urine volume and augmentation limit vary directly with variations in the body surface area, a correction factor should be introduced into the above formula in the case of individuals distinctly above or below the average adult size. As stated by the authors, no correction need be made for persons between 62 and 71 inches in height since the error involved does not exceed 5 per cent. The corrected formulas are expressed as follows:

$$Cm = \frac{U}{P} \times V \times \frac{1.73}{A},$$

$$Cs = \frac{U}{P}\sqrt{V \times \frac{1.73}{A}},$$

where A is the body surface in square meters, calculated according to the height and ideal weight, available in standard tables provided for such determinations.

Procedure (Moller, McIntosh and Van Slyke). The routine procedure advocated by the originators of this test is as follows:

The subject is not subjected to any previous routine, except that vigorous exercise is avoided. The most desirable time of day is in the hours between breakfast and lunch, at which time excretion is least liable to fluctuation. Two or three glasses of water are taken shortly before breakfast. The bladder is emptied completely, the exact time being noted. This urine is discarded. This marks the beginning of the test period, breakfast being taken at this time. Exactly one hour later the bladder is again completely emptied, the urine being saved for examination. At this time a specimen of blood is withdrawn for urea determination. Exactly one hour later the bladder is again completely emptied, the urine being saved for examination. If the urine specimens are not obtained exactly one hour apart, the exact time at which they are obtained should be noted. The volume of each specimen is measured carefully and determinations are made of the concentration of urea in the blood and urine specimens. Inasmuch as the urea is of course removed only from the plasma in the process of urine formation, plasma and not whole blood should be used for clearance determinations. However, urea exists in the water of the erythrocytes in practically the same concentration as in the plasma, so that for practical purposes in clinical studies whole blood may be employed in estimating urea clearance (but not inulin, diodrast, hippurate and other clearances). The patient remains quiet during the period of the test. The chief source of error lies in the possibility of incomplete emptying of the bladder, the liability of which is diminished by the collection of two urine specimens. In cases in which conditions are present which interfere with complete emptying of the bladder, such as prostatic hypertrophy, tumors of the uterus, advanced pregnancy, etc., the bladder should be emptied by catheter at the beginning and end of the two-hour test period. If desired, the test period may be shortened to one hour (two 30-minute urine collections), the blood sample being obtained at the mid-point.

If the urine volume (corrected) exceeds 2 cc. per minute the maximum clearance is calculated. If the urine volume (corrected) is less than 2 cc. per minute the standard clearance is calculated.

Results are expressed in terms of cubic centimeters of blood cleared of urea per minute or in terms of percentage of average normal clearance.

Example of Calculation of Maximum Clearance

Blood urea N = 14 mg. per 100 cc. = B
Urine urea N = 210 mg. per 100 cc. = U
Urine volume = 180 cc. per hour
= 3 cc. per minute = V

$$Cm = \frac{U}{B} \times V = \frac{210}{14} \times 3 = 45 \text{ cc. of blood cleared of urea per minute.}$$

$$\text{Percentage of normal function} = \frac{45}{75} \times 100 = 60 \text{ per cent.}$$

Example of Calculation of Standard Clearance

Blood urea N = 14 mg. per 100 cc. = B
Urine urea N = 420 mg. per 100 cc. = U
Urine volume = 90 cc. per hour
= 1.5 cc. per minute = V

$$Cs = \frac{U}{B} \sqrt{V} = \frac{420}{14} \times 1.22 = 36.6 \text{ cc. of blood cleared of urea per minute.}$$

$$\text{Percentage of normal function} = \frac{36.6}{54} \times 100 = 67.7 \text{ per cent.}$$

INTERPRETATION. It has been found that the maximum clearance is increased by the ingestion of caffeine, milk, a mixed meal and by small doses of epinephrine. It is decreased by the administration of pituitrin or large doses of epinephrine. In a study of the diurnal variation of the standard blood clearance, it was found to be depressed during the first hour after arising; following this, commencing before breakfast, there was a regular increase, the higher level continuing through the morning. A definite drop occurred after lunch with a subsequent rise during the late afternoon and evening. The least variation occurred during the period between breakfast and lunch (9–12 A.M.). Strenuous exercise causes a decrease in blood urea clearance, particularly in patients with impaired renal function.

The urea clearance may be abnormally low during periods of subsistence on a low protein diet. Low values have been reported during relapse in pernicious anemia and in other severe anemias. The urea clearance in pregnancy appears to remain within normal limits in subjects without severe anemia and with an adequate protein intake. Reports of low values are probably due to low protein intake. No alteration has been observed in glomerular filtration rate, renal blood flow or tubular excretory mass in normal pregnancy. Normal values are the rule in uncomplicated eclampsia and preeclampsia.

It has been observed that, particularly in patients under forty years of age, elevation of blood urea clearance (over 25 per cent above the average normal) may occur during the course of acute infections such as pneumonia and rheumatic fever. This may be due to increase in renal blood flow and glomerular filtration resulting from renal arteriolar dilatation (p. 586). The absence of this phenomenon in older patients is attributed to a decrease of renal resiliency with increasing age. An increase has been observed also after injection of pyrogens, but not in hyperthermia induced by diathermy. Erroneously low values may be obtained when the urine volume is less than 0.35 cc. per minute and erroneously high values during periods of marked diuresis. Unilateral nephrectomy is followed by an average increase of 43 per cent in urea clearance by the remaining kidney (increased blood flow) (p. 586). In children with chronic ("lipoid") nephrosis, high values (up to 200 per cent of average normal) may be obtained. This is probably due chiefly to an increase in renal blood flow, but has also been attributed, in part at least, to increase in glomerular filtration resulting from the decreased plasma colloid osmotic pressure (p. 584).

Inasmuch as all of the urea in the urine leaves the blood plasma in the glomerular filtrate, all causes of decreased glomerular filtration are potential causes of decreased urea clearance. The underlying mechanisms, which have been discussed elsewhere (p. 583), include: (*a*) diminished renal blood flow; (*b*) glomerular damage; (*c*) increased capsular pressure; and, (*d*) theoretically at least, increased plasma colloid osmotic pressure. One or more of these mechanisms are usually operative in virtually all clinically important forms of diffuse kidney disease, e.g., acute and chronic glomerulonephritis, advanced chronic pyelonephritis (bilateral), nephrosclerosis, intercapillary glomerulosclerosis, renal hypogenesis, and advanced bilateral destructive lesions (polycystic disease, tuberculosis, malignancy). They are operative also in congestive heart failure, bilateral urinary tract obstruction due to any cause, and in extrarenal conditions in which glomerular filtration and renal blood flow are seriously reduced as a result of marked hypotension (shock, Addison's disease, etc.) (pp. 583, 591).

At urine volumes of 2 cc. or more per minute (unless excessive), about 35 to 40 per cent of the filtered urea is reabsorbed (passively) in the tubules. Theoretically, therefore, decreased urea clearance could conceivably be due to excessive tubular reabsorption. This does occur in necrotizing nephroses, including so called "lower nephron nephrosis," in which conditions all components of the glomerular filtrate are returned to the blood stream in excess, unselectively, across nonfunctioning or disrupted portions of the tubule walls (p. 610). However, this mechanism apparently does not operate in acute or chronic glomerulonephritis, nor in the common forms of chronic diffuse kidney disease mentioned above. In these conditions, tubular reabsorption of urea is usually decreased.

In routine clinical practice, the urea clearance is generally the most reliable single quantitative index of the state of renal function. Its usefulness stems from several facts. The test procedure requires no intravenous injection, as do other clearance tests (inulin, PAH), the test period may be as short as one hour, and quantitative urea determinations can be readily and accurately performed in a routine clinical laboratory. Except under conditions of unusual glomerulo-tubular functional imbalance, or of very small or very large urine volumes, the urea clearance is roughly proportional to the rate of glomerular filtration (p. 582). Since this is decreased in the great majority of conditions in which renal function is impaired (p. 583), the urea clearance is usually diminished correspondingly. Moreover, not only is urea normally the most abundant urinary solid, but also it is excreted by a mechanism similar to that which is involved in the excretion of the bulk of the other important components of the urine; i.e., glomerular filtration and rather extensive tubular reabsorption, although the latter is passive in the case of urea.

In interpreting urea clearance values in disease, it must be remembered that this factor is not a precise measure of individual phases of renal function, i.e., glomerular filtration rate, renal blood flow, and tubular reabsorption or excretion. These can be measured accurately only by determination of inulin (or mannitol) and p-aminohippurate (or Diodrast) clearance, and of tubular maxima (Tm). Nevertheless, although these more refined procedures may reveal discrete abnormalities of renal physiology in the presence of essentially normal urea clearance, the latter affords a quantitative index of the efficiency of renal excretion of an important and representative constituent of the urine. It is therefore of great value in the clinical assessment of over-all renal function. Characteristic findings in disease are discussed elsewhere (pp. 614–622).

Blood Nitrogen Studies

The several conditions which may be associated with elevation of the level of nonprotein nitrogen in the blood are reviewed elsewhere (p. 166 ff.). The following discussion is concerned only with blood nitrogen retention in its relation to renal functional impairment.

In the presence of renal disease, blood nitrogen retention depends essentially upon three factors:

(*a*) The degree of renal functional impairment.

(*b*) Prerenal deviation of water.

(*c*) Excessive protein catabolism.

Since the diagnostic and prognostic significance of nitrogen retention depends upon the part played by each of these factors in any given case, they will be considered individually in greater detail.

Renal Functional Impairment. The concentration of nonprotein nitrogenous substances in the urine greatly exceeds that in the blood. Under ordinary conditions the concentration of urea in the urine is 60 times as high as in the blood, uric acid 20 and creatinine 50 times as high. The ability to concentrate these and other solid substances constitutes one of the most important functions of the kidney. The normal kidney can satisfactorily eliminate the required quantity of solids even though only relatively small quantities of water are available for their solution. Inability of the kidney adequately to eliminate nitrogenous substances may be regarded as dependent essentially upon diminution in its concentrating ability. The only method by which the kidney can compensate for this defect is by the elimination of increased quantities of water, the urine during this period of compensation being of large volume and relatively low specific gravity.

During this compensatory stage, in spite of the presence of renal functional impairment, the blood nitrogen values may remain within essentially normal limits. They rise in the absence of extrarenal factors only when the renal lesion has progressed to the point at which the kidney fails to eliminate sufficient water to compensate for the diminution in its power of concentration. Under such circumstances the urine is of consistently low specific gravity regardless of the volume of water eliminated. The variable significance of nitrogen retention dependent entirely upon renal functional insufficiency will be considered in the discussion of various types of renal disease.

Prerenal Deviation of Water. Since, particularly in the presence of renal disease, the ability of the kidneys to eliminate nonprotein nitrogenous elements depends largely upon the amount of water available for excretion by the kidneys, blood nitrogen retention may be induced by factors which cause prerenal deviation of water. The most important of these may be classified under two headings: (1) those producing edema, and (2) those resulting in dehydration.

(1) EDEMA. Water retention in the tissues, due to any cause, imposes a burden upon the nitrogen-excretory function of the kidneys, particularly if their concentrating ability is already impaired. This factor plays an important part in the production of nitrogen retention in acute nephritis. The occurrence of myocardial insufficiency with edema, so frequently a complication of chronic hypertensive nephritis, may precipitate renal insufficiency in an individual with a previously well compensated renal functional defect. The same phenomenon may be observed in association with edema due to a nephrotic lesion or to malnutrition with hypoproteinemia complicating chronic nephritis.

(2) DEHYDRATION. Excessive loss of water or water privation exerts a similar influence. Protracted vomiting, so common a feature of advanced nephritis, and, less commonly, profuse diarrhea, may result in nitrogen retention in patients with previously compensated renal functional impairment. Excessive perspiration or vaporization of water from the body surface in complicating febrile disorders may act in a similar manner.

Excessive Protein Catabolism. With a constant state of renal functional activity, an excessive rate of protein catabolism will cause a tendency toward the accumulation of nitrogenous elements in the blood. This factor may play a significant role in the development of nitrogen retention in nephritis. Among the more common conditions associated with increased protein destruction and a consequent increase in the quantity of nonprotein nitrogenous substances that must be eliminated by the kidneys are the following:

(1) HIGH PROTEIN INTAKE. Under conditions of normal renal function, normal water balance and adequate fluid intake, the ingestion of large quantities

of protein is followed by prompt elimination of the excess nitrogen with no consequent significant elevation of the level of nitrogen in the blood. However, if renal function is impaired, and especially if, in addition, the urine volume is diminished, the nonprotein nitrogenous elements in the blood may increase during periods of high protein intake and return to normal following the administration of a diet low in protein.

(2) INFECTIONS. Infectious processes, occurring in patients with renal functional impairment, may result in an increase in blood nitrogen. Infections such as tuberculosis, even in the absence of fever, cause toxic destruction of protein. In febrile disorders, increased protein catabolism results from both toxic destruction and increased energy requirement incident to the elevation of body temperature. The tendency toward nitrogen retention in such conditions is aggravated by the frequent coexistence of other factors exerting a similar influence, such as deficient fluid intake, excessive evaporation from the skin and respiratory tract, diarrhea, vomiting, edema, and so on. Excessive protein catabolism may perhaps contribute to the nitrogen retention frequently observed in acute nephritis and bichloride nephrosis.

(3) DEHYDRATION. Dehydration due to water privation, vomiting, diarrhea, or the like, so alters tissue metabolism as to result in an increase in the rate of protein catabolism. The increase in the level of blood nitrogen which occurs under such circumstances is due to the combined effects of this factor, diminution in the quantity of water available for elimination by the kidneys, and inadequate renal blood flow.

(4) INCREASED CATABOLISM OF PROTEIN. This occurs in uncontrolled diabetes mellitus, thyrotoxicosis, adrenal cortical hyperfunction, pituitary basophilism, infectious diseases, malignancy, etc.

Obviously, the interpretation of the significance of blood nitrogen retention in individuals with renal disease depends upon the proper evaluation of the relative importance of renal and extrarenal factors in its production.

Relative Degree of Retention of Individual Nitrogenous Elements in Renal Disease. UREA. Urea nitrogen, representing normally approximately one half of the total nonprotein nitrogen of the blood, usually constitutes by far the largest proportion of elevated blood nitrogen values. In individuals with 200 mg. per 100 cc. of nonprotein nitrogen, urea nitrogen may constitute 80 to 90 per cent of the total. In some instances, however, the undetermined fraction may be increased relatively more than urea, although such findings are uncommon.

URIC ACID. Early observers believed that, in the presence of renal failure, the uric acid concentration in the blood increases before that of urea and creatinine. They inferred from this observation that uric acid is normally eliminated with less facility than are the other nitrogenous elements and hence is the first to be retained in the blood under conditions of renal functional impairment. This hypothesis has not received general confirmation, and, although the blood uric acid is usually increased in renal insufficiency, it is far less satisfactory as an index of the degree of functional impairment or even of the presence of renal disease than is the blood urea concentration. Patients with gout, arterial hypertension and other conditions (see p. 200) may exhibit elevations in blood uric acid in the absence of renal disease.

Creatinine. The belief has been widely held by clinicians that creatinine is more readily eliminated by the kidney than urea, and increases in concentration in the blood only when renal function is seriously impaired; hence creatinine retention has a serious prognostic significance (normal 1–2 mg. per 100 cc.). Whereas it is commonly observed that in renal insufficiency the blood urea concentration may be considerably increased for long periods of time before any significant increase in creatinine occurs, the explanation indicated above has not been substantiated. There can be no question, however, of the grave import of high blood creatinine values. The variable significance of creatinine retention in various types of renal disease will be considered below.

Ammonia, Amino Acids, Undetermined Nitrogen. Blood ammonia is not increased in renal insufficiency, nor are amino acids, in most instances. Occasionally, however, the amino acid content of the blood may rise in the late stages of uremia, due perhaps to excessive protein catabolism or to acute hepatic necrosis, which may occur in that condition. The undetermined nitrogen fraction is usually elevated in proportion to the increase in total nonprotein nitrogen. In a few instances, however, relatively high values have been observed in uremia. The significance of this fraction has not been determined.

Blood Nonprotein Nitrogen in Renal Disease. Nitrogen retention in renal disease depends upon the nature and extent of the renal lesion and upon extrarenal factors, chiefly prerenal deviation of water and excessive protein catabolism. Since the relative importance of each of these factors may vary in different types of renal disease, the significance of blood nitrogen retention must vary accordingly.

Acute Glomerulonephritis. Increased blood nitrogen values are frequently observed in acute nephritis, the degree of retention varying from slight elevations (e.g., NPN 45 mg., Urea N 25 mg., Creatinine 2 mg. per 100 cc.) to extremely high figures (e.g. NPN 200 mg., Urea N 160 mg., Creatinine 25 mg. per 100 cc.). Nitrogen retention in this condition is the resultant of several factors, including (*a*) impaired renal function, (*b*) prerenal deviation of water, and (*c*) excessive protein catabolism and/or diminished anabolism.

There is considerable variation in the degree of renal functional impairment in acute glomerulonephritis. In some cases renal function is essentially intact. In others, it is markedly diminished (p. 616). The majority exhibit some decrease in glomerular filtration and, to a lesser extent, and less consistently, in renal blood flow. This, in itself, is not always sufficient to cause the marked elevations of blood nitrogen which are frequently observed and which are dependent upon the operation of extrarenal factors superimposed upon a relatively mild renal functional defect. In some cases of acute, and also chronic, glomerulonephritis, one of the factors contributing to the development of renal functional impairment is obstruction of glomeruli and tubules by accumulations of fibrin and other protein coagula.

Oliguria is a common feature of acute nephritis. Whereas in a relatively small proportion of cases this is a manifestation of renal functional impairment (glomerular occlusion), it is usually dependent upon prerenal deviation of water, due to edema, vomiting, diarrhea, or fever. The edema of acute nephritis is probably not dependent primarily upon the renal lesion per se but rather upon generalized capillary injury with consequent increased permeability, upon a diminution in the concentration of plasma proteins, or upon myocardial weakness (p. 315). Edema may be latent or frank; as much as 4000 cc. of water may be retained without being detectable by ordinary methods of physical examination.

This prerenal deviation of water is an important factor in determining the presence or degree of blood nitrogen retention in acute nephritis. Occasionally edema due to myocardial insufficiency may exert an influence in this connection as may, more frequently, limitation of fluid intake and active purgation, which are common practices in the presence of edema.

Acute nephritis should be considered as a local manifestation of a generalized process affecting the tissues and capillaries throughout the body. It is often accompanied by increased protein catabolism and/or decreased protein anabolism (synthesis). In fact, this feature may be so pronounced that an increase in urinary nitrogen and a negative nitrogen balance may be present simultaneously with an increase in the nonprotein nitrogen content of the blood.

It is evident, therefore, that several factors must be considered in determining the prognostic significance of high blood nitrogen values in acute nephritis. In most cases the condition is a temporary one, the nitrogen values returning to normal upon subsidence of the acute infection. Occasionally, with improvement in the clinical manifestations, a transient rise in blood nitrogen may occur during periods of diuresis and diminution in edema. Nitrogen retention associated with urine of a low specific gravity, indicating renal functional impairment, is much more significant than that associated with urine of a high specific gravity. Because of the essential character of the condition, with its inherent capabilities of retrogression and eventual recovery, anatomical and functional, high nitrogen values have less prognostic significance in acute than in chronic nephritis. This is particularly true if they are dependent largely upon extrarenal factors, as is commonly the case. Despite these complicating circumstances, analyses of large groups of cases reveal that the prognosis for complete recovery from acute glomerulonephritis is generally not as good in subjects with severe azotemia as in those with blood urea N values below 30 mg./100 cc. This correlation is by no means consistent, however, exceptions occurring in both directions.

The proper interpretation of the retention of nonprotein nitrogen in the blood in acute, as indeed in chronic nephritis, depends upon the relative part played by the renal lesion per se, whether it subsides or progresses into chronic nephritis, and upon the relative importance of extrarenal factors. Single determinations are less valuable than serial determinations because of marked temporary variations due to extrarenal influences. In the majority of cases nitrogen retention may be regarded as a result of the combined effects of mild or moderate renal functional impairment, prerenal deviation of water and excessive protein catabolism, the last two factors being often more important than the first. Even advanced grades of nitrogen retention in acute nephritis may possess relatively little significance from the standpoint of the estimation of the extent of renal damage. They must be interpreted in the light of information afforded by other tests of renal functional efficiency, particularly the concentration and clearance tests, which are relatively uninfluenced by extrarenal factors.

Chronic Glomerulonephritis. The factors involved in the production of azotemia in chronic nephritis are essentially the same as those discussed in connection with acute nephritis with the significant exception that the pathologic process in the kidneys is chronic and progressive and that, as a result, renal functional impairment plays a more important and extrarenal factors a less important part than in acute nephritis. For a variable period of time, depending upon the rapidity of progression of the renal lesion, the blood nonprotein nitrogen may remain within normal limits. This is almost invariably the case during the so-called latent period, although occasionally the progress of the disease may be halted temporarily at a more advanced stage of kidney damage, with mild azotemia. The blood nonprotein nitrogenous components are usually within normal limits also in the so-called "nephrotic," i.e., hypoproteinemic edematous (chronic active) stage of chronic glomerulonephritis. In this connection, it must be remembered that although blood urea N (normal 8–23 mg./100 cc.) and NPN (normal 16–40 mg./100 cc.) concentrations within the rather wide normal range must be regarded as normal, values for urea N above 15 mg./100 cc. and for total NPN above 35 mg./100 cc. are often in reality abnormal. During these relatively nonazotemic periods, abnormal retention of urea is minimized owing to the fact that the diminished ability of the kidneys to excrete this substance (and others) in adequate concentration is compensated, more or less successfully, by excretion of larger volumes of urine (polyuria), except in edematous phases.

As the kidney lesion progresses and renal functional efficiency becomes more distinctly impaired, a point is reached where increased water elimination is no longer able to compensate for the increasing inability to excrete solids. Increasing retention of nonprotein nitrogen ensues and, at first mild, progresses steadily but with variable rapidity, until in the terminal stages (uremia) extremely high levels may be reached. In patients suffering with this form of the disease, nonprotein nitrogen values of more than 100 mg. per 100 cc., urea nitrogen of 80 mg. and creatinine of 5 mg. per 100 cc. foreshadow a rather speedy fatal termination. Blood urea N values above 900 mg./100 cc. have been reported. Urea usually constitutes the largest proportion of this increase in nonprotein nitrogen (60–90%), the blood creatinine commonly remaining within normal limits for a considerable period of time during which the urea nitrogen concentration has exhibited a progressive increase.

As in the case of acute nephritis, marked elevations of blood NPN frequently occur during the course of chronic nephritis due to the superimposition of extrarenal factors. Under such circumstances, being not necessarily indicative of the actual extent of the renal lesion, nitrogen retention loses its serious significance. As a rule, the course of the disease is punctuated by periods of excessively high blood nitrogen values which subside after correction of extrarenal influences. Among the most common of these are intercurrent infections, excessive vomiting and diarrhea, excessive protein ingestion, water privation, acute exacerbations of the renal process, and congestive heart failure. These factors have been considered in detail above; they operate largely in one or both of two ways, i.e., increased protein catabolism and prerenal deviation of water. Thus, renal insufficiency is precipitated by the imposition of an excessive burden upon a diminished renal functional reserve. Upon removal or cessation of operation of these factors the blood nitrogen concentration falls to the level determined by the degree of renal functional impairment and the efficiency of the compensating mechanism (polyuria).

It must be apparent that too much reliance must not be placed upon the concentration of the nonprotein nitrogenous constituents of the blood as an indication of the extent of renal damage in chronic nephritis. In the absence of complicating conditions the pathologic process in the kidneys may be rather far advanced before the blood urea N (or NPN) rises above the upper limit of normal; in the late or terminal stages it may have some prognostic significance, particularly if creatinine retention is marked. However, at any time during the course of the disease, advanced grades of nitrogen retention may occur because of the operation of one or more of the several extrarenal factors mentioned above. In the interpretation of high nonprotein nitrogen values, these complicating influences must be carefully considered and their part in the production of the existing condition properly evaluated.

NEPHROSCLEROSIS. The concentration of nonprotein nitrogen in the blood is often within normal limits during the entire course of essential hypertension and nephrosclerosis. Nevertheless, even in early stages, the presence of abnormality of renal function (glomerular dynamics) is evidenced by decrease in glomerular filtration and reduction in renal blood flow (p. 586). Statements made above concerning the difficulty of interpreting blood urea N and NPN values in the upper range of normal in glomerulonephritis apply equally here. In the late stages of this condition, and in so-called "malignant hypertension," renal failure may occur and progress rapidly to a fatal termination. Because of the frequent occurrence of vascular accidents or cardiac failure incident to the prolonged state of hypertension, renal insufficiency is observed less frequently than in chronic glomerulonephritis. However, if it does occur, the metabolic features and blood chemical findings are identical with those which characterize the latter condition.

DESTRUCTIVE RENAL LESIONS. Under this heading may be considered such conditions as renal tuberculosis, malignancy, pyelonephritis, hydronephrosis, polycystic disease, etc. The estimation of renal functional efficiency in these disorders is of particular importance from the standpoint of the advisability of surgical intervention and the determination of the operative procedure to be employed.

Since adequate elimination may be carried on by two thirds of one kidney, the presence of nitrogen retention in destructive lesions of the kidney indicates extensive renal damage. It may be interpreted as signifying either that both kidneys are involved in the pathologic process or that the uninvolved kidney is overburdened by the necessity of eliminating excessive quantities of nitrogen resulting from increased toxic destruction of protein. In this group of disorders the influence of extrarenal factors must be carefully evaluated. High protein intake, fever, water privation, vomiting, edema, diarrhea, etc., must be taken into consideration.

The consensus is that little information of significance in prognosis can be gained from single nonprotein nitrogen determinations. In destructive renal lesions it is the persistence rather than the degree alone of nitrogen retention to which attention should be directed and by which the extent of renal damage should be estimated. The persistence of elevated blood nonprotein nitrogen values in such cases following the institution of proper therapeutic procedures is of definitely grave prognostic import. Because of the fact that individuals with marked nitrogen retention are poor operative risks, repeated preoperative estimations of the nonprotein nitrogen concentration of the blood are of value in determining the time at which radical surgical measures may be attempted with a minimum of risk to the patient. In surgical disorders of the kidneys, the functional efficiency of each kidney should be studied by estimating its capacity for dye elimination (p. 612), either by ureteral catheterization or by direct vision through the cystoscope.

NECROTIZING NEPHROSES. Acute renal failure, with azotemia, occurs in the presence of severe degenerative changes (necrosis) in the renal tubular epithelium. From the standpoint of etiology, there are two main classes of conditions in this category: (1) those due to nephrotoxic poisons, e.g., mercury, uranium, carbon tetrachloride, sulfonamides, chlorate, etc.; (2) those due fundamentally to severe renal ischemia (p. 322), e.g., shock, "lower nephron nephrosis," etc. From the standpoint of pathological physiology, there seems to be little justification for separating these two groups. Moreover, the term "lower nephron nephrosis" (severe trauma, burns, mismatched transfusions, postoperative, etc.) is misleading, since the implication that the necrosis in this condition is restricted to the distal, and toxic nephrosis to the proximal segment of the tubule is not entirely correct.

In these conditions, severe oliguria (or anuria) and azotemia develop because of removal (necrosis, desquamation) of portions of the selective barrier to the free passage of components of the glomerular filtrate back into the blood stream. As a result of disruption of continuity of this functional (and morphological) barrier, excessive amounts of all components of the filtrate diffuse back, unselectively (p. 596). In the conditions in which arterial hypertension is a dominant feature (shock syndrome, "lower nephron nephrosis"), there is also a diminished volume of glomerular filtrate (and decreased renal blood flow), which contributes to the inadequacy of renal function. In the past, the terms, "renal crush syndrome," "hemoglobinuric nephrosis," "gestation nephrosis," "hepatorenal syndrome," etc., have been applied to acute tubular necrosis and renal failure occurring under circumstances implied by each of these designations. In some cases, i.e., "crush syndrome" and hemoglobinuric nephrosis (e.g., mismatched transfusion, blackwater fever), it has been suggested that factors other than ischemic necrosis may be involved, viz., obstruction of tubules by precipitated myoglobin or hemoglobin (especially if urine is highly acidic), and toxic effects on the kidney of circulating methemoglobin and myoglobin. At the present time, it is generally believed that severe hypotension, with intrarenal vasoconstriction and renal ischemia, is the most important etiologic factor in this important group of conditions.

In some instances, azotemia occurs in the absence of significant oliguria. The combination of moderate decrease in glomerular filtration, renal blood flow, and tubular concentrating functions, and increased protein catabolism (severe trauma, operations, burns, etc.) may result in inadequate excretion of urea (and other urine solids) despite urine volumes of 800 to 1200 cc. (low specific gravity). Azotemia may also persist during periods of recovery from necrotizing nephroses, even in the presence of polyuria (with hyposthenuria), due to functional inadequacy of the newly regenerated renal epithelium (p. 596).

URINARY OBSTRUCTION. In patients with prostatic or urethral obstruction or with bilateral ureteral calculus, and in some cases of unilateral ureteral calculus (with "reflex" anuria), the back pressure may be so great that effective filtration through the glomerular membrane cannot occur. Under such circumstances, remarkable grades of nitrogen retention may be observed. It is believed by some that in these conditions the blood creatinine frequently remains normal in the presence of extremely high urea nitrogen values, in contradistinction to the findings with similar grades of urea nitrogen retention in nephritis. In the experience of the authors, this is not generally the case. In fact, the reverse is apparently more commonly observed; i.e., with equal grades of urea retention, blood creatinine values are higher in urinary obstruction than in nephritis.

In the absence of permanent renal disease, relief of the obstruction and institution of adequate drainage are usually followed by restoration of the normal blood nitrogen level. This may occur in the presence of extremely high nitrogen values. In a patient with prostatic obstruction, with a total blood nonprotein nitrogen concentration of 420 mg. per 100 cc. and creatinine 32 mg. per 100 cc., following prostatectomy in two stages the nonprotein nitrogen fell to 54.8 mg. and the creatinine to 2.6 mg. per 100 cc. Nitrogen retention in urinary obstruction amenable to correction does not possess the significance attributed to it in primary kidney disease.

Urea in Other Body Fluids

Because of its extreme diffusibility, urea exists in the lymph, spinal fluid, bile and pancreatic juice in practically the same concentration as in the blood. Considerable interest has been attached to the investigation of the nonprotein nitrogen and urea contents of the sweat because of the possibility of stimulating elimination through this channel in patients with renal insufficiency. It has been found that the nonprotein nitrogen content of the sweat of normal individuals averages about 30 per cent greater than that of the blood, about 65 per cent of the total NPN consisting of urea nitrogen. Whereas under ordinary circumstances the amount of nitrogen lost through the skin is quite small, it may be greatly increased if perspiration is profuse. However, it is doubtful that this channel of elimination can be used to practical advantage in patients with nitrogen retention since the coincident elimination of relatively large amounts of water by the skin may tend to increase rather than to decrease the level of blood nitrogen. The significance of the concentration of the various nonprotein nitrogenous substances in the cerebrospinal fluid has been considered elsewhere (p. 633).

The urea content of saliva is approximately 80 per cent of that of the blood. The combined urea and ammonia nitrogen content of the saliva more closely approximates the concentration of urea nitrogen in the blood because of the fact that urea in the saliva is rather readily broken down into ammonium carbonate through bacterial action. Because of the mercury binding capacity of urea, determination of the mercury combining power of saliva has been suggested as an index of the degree of renal functional insufficiency in cases in which determination of the blood urea nitrogen concentration is not practicable. It has been found that 100 cc. of saliva normally contain enough urea to combine with 30 to 50 cc. of a 5 per cent solution of mercuric chloride. This constitutes the mercury combining index or the salivary index. It is stated that the probable blood urea concentration may be roughly calculated from the salivary index as follows:

$$1.43 \times \text{salivary index} - 34 = \text{probable blood urea in mg. per 100 cc. of blood.}$$

Presumably, when the salivary index is below 50 there is no retention of urea nitrogen in the blood in 90 per cent of cases; in 10 per cent there may possibly be a mild degree of retention but the blood urea concentration is never above 60 mg. per 100 cc. under such circumstances. This method is by no means accurate and should be used only as a preliminary diagnostic procedure and never as a substitute for the determination of blood urea nitrogen.

ELIMINATION OF FOREIGN SUBSTANCES

The kidney normally possesses the ability to eliminate certain foreign substances from the blood stream. This fact has served as the basis for many tests of renal function, various substances being used, including potassium iodide, methylene blue, fuchsin, lactose, indigo-carmine, and, most widely employed, phenolsulfonphthalein.

The Phenolsulfonphthalein Test. Rowntree and Geraghty, in 1912, introduced the use of phenolsulfonphthalein as a test of renal functional efficiency. This dye is promptly and almost completely eliminated in the urine. The PSP test is one of the most widely employed measures for the estimation of renal functional capacity.

The bladder having been emptied, the patient drinks 600 cc. of water. One cubic centimeter of phenolsulfonphthalein solution, containing 6 mg. of the dye, is injected intravenously 30 minutes later with the patient at rest. Urine is collected 15, 30, 60 and 120 minutes after the injection. The 15-minute specimen alone is sufficient for routine practical purposes. Each specimen of urine is diluted to a suitable volume, sodium hydroxide (10 per cent solution) is added until a maximum red color is developed, and the amount of dye (PSP) determined by comparison with a standard solution containing 6 mg. of PSP in 1000 cc. of water (alkalinized). It is, of course, essential that there be no bladder retention of urine and that the bladder be completely emptied at the end of each period.

Normal excretion values are as follows:

Specimen	%	Average %
15-minute	28–51	35
30-minute	13–24	17
60-minute	9–17	12
120-minute	3–10	6
total 2-hour	63–84	70

Although flattening of the curve of elimination is of some significance, elimination of less than 25 per cent of the dye during the first fifteen minutes (30 per cent in twenty minutes) is the most significant evidence of renal functional impairment yielded by this test. If the bladder cannot be emptied at 15 minutes, the 30 minute sample should normally contain not less than 40 per cent of the administered dye.

In a less satisfactory procedure, the dye (6 mg.) is injected intravenously or intramuscularly (lumbar region) and the bladder is emptied one hour and two hours later, allowing ten additional minutes in the case of intramuscular injection. Normal subjects excrete 40 to 60 per cent of the dye in the first hour and 20 to 25 per cent in the second hour. However, especially with intramuscular injection, values above 50 per cent in the two-hour period must be regarded as within normal limits. Mild functional impairment is not revealed by this procedure as readily as by the fractional test outlined above. Inability to excrete a normal amount of the dye during the first fifteen minutes, due to inability to achieve a high concentration in the urine, may be obscured by prolonging the period of collection of urine to one hour. Abnormal results have been obtained with the fractional procedure in as many as 30 per cent of subjects with mild renal disease in whom normal results were obtained by this method (one- and two-hour collections).

Certain facts must be kept in mind in interpreting results obtained by the phenolsulfonphthalein test. Low values may be obtained in the presence of factors which tend to cause prerenal deviation of water. Whereas in normal individuals the quantity of dye eliminated is independent of the urine volume, in the presence of severe impairment of renal function the output of phenolsulfonphthalein varies more or less directly with the volume of the urine and can be increased by liberal administration of water. As is the case with clearance tests, PSP excretion is lower (10 to 15 per cent) in the erect than in the recumbent position This is due presumably to the intrarenal (and general intra-abdominal) vasoconstriction which occurs on assuming the erect position. This postural difference in PSP excretion is exaggerated in subjects with glomerulonephritis and nephrosclerosis.

The average normal value for PSP clearance is about 400 cc., which indicates that the largest proportion of the dye is removed from the blood plasma by tubular excretion and a comparatively minor fraction by glomerular filtration. Consequently, excretion of phenolsulfonphthalein is diminished more directly by specific impairment of tubular excretory capacity (tubule cell damage) and reduction in renal blood flow than by primary glomerular damage or reduction in glomerular filtration out of proportion to decrease in effective renal plasma flow. The fundamental differences in the mechanisms of excretion of urea and PSP account for discrepancies observed occasionally between urea clearance and PSP excretion values in certain cases of renal disease or other conditions (e.g., congestive heart failure) affecting renal function. Stated in general terms, urea clearance is primarily a reflection of glomerular filtration and PSP excretion a more direct reflection of tubular excretory function and effective renal plasma flow. However, because of the physiological interrelationship between these factors and because of the usual involvement of all of them, although not to the same extent, in the majority of clinical disorders affecting kidney function, there is generally, although not invariably, good qualitative if not quantitative agreement between values for PSP excretion and urea clearance.

Under normal conditions a certain proportion of the injected phenolsulfonphthalein is removed from the blood stream by the liver. In patients with impaired hepatic function this fraction is diminished, the urinary excretion of the dye being correspondingly increased. Consequently, in cases in which hepatic disease is present together with renal disease, the urinary elimination of phenolsulfonphthalein may be greater than if the liver function were normal. Under such circumstances, borderline normal results may be misleading.

This test may be employed to determine the functional capacity of each kidney. This is one of its most useful applications. Ureteral catheters are introduced and the test performed as outlined above. In normal individuals the dye appears from both sides usually in three to five minutes following intravenous injection. An approximately equal amount is eliminated by each kidney. In the presence of unilateral renal disease the appearance time is delayed on the diseased side and the quantity eliminated is diminished in proportion to the degree of renal functional impairment. It is important to keep in mind the fact that the opposite kidney, if functioning normally, should excrete not only the 50 per cent of the total normally excreted by both kidneys, but also that portion not eliminated by the diseased kidney.

Localization of Renal Functional Defect

As indicated in the discussion of clearance tests (p. 581), it is possible, by currently available methods, to measure certain discrete phases of renal function, e.g., glomerular filtration rate, effective renal blood flow, and maximum tubular capacity for excretion and reabsorption. This can be done with satisfactory precision in normal subjects and in the majority of disease states. It must be remembered, however, that interpretations of inulin clearance values in terms of glomerular filtration rate are invalid under conditions of extensive back-diffusion of components of the filtrate across damaged tubule walls (p. 584). Interpretations of PAH or Diodrast clearance values in terms of effective renal plasma flow are invalid under conditions of subnormal extraction of these substances from the blood plasma (low extraction ratios, p. 587). The latter source of error can be eliminated by determining the extraction ratio (renal vein catheterization). These procedures and concepts have contributed enormously to present understanding of normal and pathological physiology of the kidney, and no other available test procedures can approach them in accuracy of measurement of the functions in question. In no other way can the factors involved in the production of renal functional impairment in a given case be dissected and their relative contribution to the deficiency evaluated. However, because of technical difficulties, the use of these methods is almost necessarily restricted to specialized clinics and laboratories, and they cannot readily be employed as routine diagnostic procedures in general practice nor in the average laboratory. Fortunately, the type of information usually required by the clinician may be obtained satisfactorily by methods that are readily applicable in routine practice. The relations of these clinically available procedures to various aspects of renal function are indicated in Table 42. The studies used most extensively in the investigation of renal function are the urea clearance, phenolsulfonphthalein excretion, and concentration tests, and the blood urea N (or NPN) concentration. Although the findings with these procedures may vary considerably, certain patterns of abnormality are exhibited in common types of kidney disease that reflect the nature of the functional aberration and, to a certain extent, of the morphological lesion.

Table 42. Functional significance of procedures commonly employed in kidney disease.

FUNCTIONS	SPECIFIC STUDIES	CLINICAL TESTS
Glomerular filtration	Inulin clearance Mannitol clearance	Urea clearance
Renal plasma flow	PAH clearance Diodrast clearance	PSP excretion
Proximal tubule activity	Excretion-PAH Tm Absorption-glucose Tm	PSP excretion
Distal tubule activity	Concentration Dilution Electrolyte balance Acid-base balance Ammonia formation Urine acidification	Concentration test Dilution test Serum electrolytes Serum CO_2 content Serum pH Urine ammonia Urine titratable acidity Urine pH

Acute Glomerulonephritis (Table 41, p. 585). This is characterized primarily by inflammatory changes in the glomeruli, which are engorged, and many of which are blocked, with consequent reduction in filtration surface area and, therefore, in filtration rate. Reduction in the latter is probably contributed to also by the increased interstitial pressure in the kidneys, which decreases the blood flow and increases the capsular pressure (p. 583). The relatively greater decrease in filtration rate than in effective renal plasma flow results in decrease in the filtration fraction (p. 588). The impaired glomerular function is reflected in abnormally low values for inulin clearance and urea clearance in all but very mild cases. This is probably due in part also to the fact that slowing of filtration prolongs the period of contact of the smaller volume of filtrate with the tubular reabsorptive surface, permitting increased reabsorption of components of the filtrate (water, electrolytes, urea, inulin, etc.). In the erect position, values for inulin, urea, and PAH clearances are considerably lower than in recumbency. This phenomenon, indicative of decreased filtration and renal blood flow, is due to intrarenal vasoconstriction, the renal vascular adjustment to orthostasis. The associated decreases in urine volume and in electrolyte and urea excretion predispose to the increase in edema and nitrogen retention in patients with acute nephritis who are not confined to bed.

In the great majority of cases of acute nephritis the blood urea clearance diminishes during the first two months, reaching values of 50 per cent or less of normal. Occasionally, however, essentially normal values may be maintained throughout the acute stage of the disease. Prognosis as to eventual recovery appears to depend, not so much upon the absolute values observed during the early stage, as upon the duration of the period of impaired renal function. It has been observed that recovery occurs in those cases in which a consistent rise in blood urea clearance values, progressing to within normal limits, begins within four months after the acute onset of the condition. In those cases in which no such tendency is noted within six months, the condition almost invariably progresses to the chronic or terminal stages of nephritis with eventual renal insufficiency. Low values during the first four months of acute glomerulonephritis are not at all inconsistent with complete recovery, both functional and anatomical. During this period, therefore, the urea clearance is of little value in determining the eventual outcome of the condition, since progression into chronic glomerulonephritis may occur in cases with normal clearance values during the acute stage of the condition.

In the initial stages, except in fulminating cases, there may be little indication of tubular malfunction. The urine specific gravity is often high and PSP excretion may be essentially normal, even in the presence of increase in blood urea N. This is a reflection of dissociated impairment of glomerular function. The urine volume is characteristically low, due primarily to decreased filtration and excessive reabsorption, as indicated above, and in part also, in some cases, to prerenal deviation of water (e.g., vomiting). With increasing duration or severity of the disease, degenerative changes invariably develop in the tubules (inadequate blood supply, p. 562). This is reflected in decreases in PAH Tm and PSP excretion, and in the appearance of hyposthenuria (p. 597). At this stage, low values are usually obtained for PAH or Diodrast clearance, suggesting decrease in renal blood flow.

However, these are largely attributable to low extraction of these substances, due presumably to the decreased tubular function (p. 562). If these clearance values are corrected for the diminished extraction ratio (p. 587), the findings suggest an essentially normal or even increased blood flow to the functioning tubular tissue. Viewed in conjunction with the data on glomerular filtration and PAH Tm, these observations may be interpreted as signifying glomerular and tubular damage, the former predominating, with active hyperemia of the functioning nephrons.

The occurrence of blood nonprotein nitrogen retention (NPN, urea N) in acute glomerulonephritis is discussed elsewhere (p. 606), and requires only brief mention here. It is the general experience that significant elevation of blood urea N, i.e., above the upper limit of normal (20–23 mg./100 cc.), is often not observed in acute (and chronic) nephritis until the urea clearance has fallen below 50 per cent, and in some instances to 20 per cent of the average normal. However, it must be recognized that any significant decrease in urea clearance, other things being equal, must inevitably result in abnormal retention of urea N in the body. Inability to detect this retention is due to inability to interpret as abnormal any values for blood urea N within the normal range. It should be pointed out, too, that at a plasma urea N of 40 mg./100 cc. and a urea clearance of 35 cc. (50%), the kidneys are excreting as much urea as at a plasma level of 20 mg./100 cc. and a urea clearance of 70 cc. (100%). Variation in blood urea N due to extrarenal factors must also be borne in mind (e.g., diet, protein catabolism, vomiting, diarrhea, edema, etc.).

The inherent reversibility of the renal lesions is indicated by the fact that all functional defects may disappear, and usually do so, in some cases very rapidly. They persist in cases which progress to chronic glomerulonephritis, or those which proceed to a fatal termination in the acute stage.

During the period of recovery, values for urea clearance and blood urea N return to normal usually before those for PSP excretion and maximum concentrating capacity (concentration test). Hyposthenuria may persist for weeks or months after complete subsidence of the acute renal lesion, as indicated by absence of symptoms and urine abnormalities, and normal clearance values. This is attributed to temporary functional inadequacy of the newly regenerated tubular epithelial cells.

Chronic Glomerulonephritis (Table 41, p. 585). The clinical and laboratory manifestations of chronic glomerulonephritis vary widely in different patients and at different times in individual cases. This is due mainly to the fact that the progress of the disease is characterized by stages exhibiting different anatomical lesions and functional abnormalities (and clinical manifestations), the duration of which varies markedly in different subjects. These are commonly designated as (1) latent, (2) chronic active, or nephrotic, and (3) terminal.

LATENT STAGE. As the term implies, this is a period during which the renal lesion is not progressing actively (temporary arrest). This usually occurs immediately following an attack of acute glomerulonephritis, and the manifestations therefore depend upon the condition of the kidneys at the time of transition from the acute to the chronic state. The usual functional findings are: (1) decreased inulin and urea clearances (decreased glomerular filtration); (2) decreased PAH clearance and PSP excretion (decreased renal plasma flow and/or decreased tubular excretion); (3) hyposthenuria. Glomerular filtration (inulin and urea clearances) is characteristically diminished relatively more than PSP excretion. These abnormalities may be of minimal degree; their extent and the presence or degree of azotemia vary considerably and, as indicated above, depend upon the severity of glomerular and tubular damage at the moment. The chronic process, although fundamentally progressive in nature, may become arrested temporarily, with a variable period of latency, at any time, except perhaps in the terminal stage.

NEPHROTIC STAGE (CHRONIC ACTIVE). This is characterized by oliguria, marked albuminuria, hypoproteinemia (p. 156), hypercholesterolemia (p. 115), and edema. Despite the uniformity of the clinical picture and these urinary and blood findings, the results of studies of renal function vary widely (Table 41, p. 585). As in the case of the latent stage, this is due mainly to differences in the rate of progress of the kidney lesions in different cases and at different times in individual cases.

All measurable functions are usually depressed, glomerular filtration (urea clearance) generally more than renal blood flow (PAH clearance). The PAH Tm is rather consistently decreased, reflecting the tubular damage. As in acute nephritis, the ratio of PAH clearance to PAH Tm, i.e., the blood flow per unit of functioning nephrons, is greater than normal, implying a state of hyperemia of these nephrons. PSP excretion is decreased and concentrating ability is impaired (hyposthenuria). All of these functions deteriorate as the disease progresses, with increasingly abnormal findings with all renal function tests, and, usually, increasing azotemia.

TERMINAL STAGE (Table 41, p. 585). Transition into the terminal stage may be identified, for purposes of convenience, by a fall in urea clearance to values below 20 per cent of the average normal. Obviously, in this stage, there is severe impairment of all functions of the kidney, with isosthenuria and increasing azotemia. The clinical syndrome of uremia appears, as well as other evidences of renal failure, e.g., dehydration (p. 622) and acidosis (p. 623). The more precise tests of renal function, including clearance procedures and the PSP tests, are almost never required, and are of academic interest almost exclusively. The progress of the disease is reflected satisfactorily for practical purposes during this terminal period in such factors as the blood urea N concentration, serum CO_2 content, anemia, etc.

Necrotizing Nephroses (Acute Renal Failure). The pathological physiology of this group of conditions is discussed elsewhere (p. 610). They are characterized by extensive necrosis of tubular epithelial cells, due either to nephrotoxic agents (e.g., mercury), or to ischemia resulting from acute depression of renal blood flow, associated usually with the shock syndrome ("lower nephron nephrosis").

In severe cases, in the initial stage of anuria or marked oliguria, virtually all of the modified glomerular filtrate is returned to the blood plasma by back-diffusion, and, obviously, all clearance studies yield extremely low or zero values. What little urine may be excreted has a specific gravity of about 1.010, and the blood urea N increases progressively.

In cases which ultimately recover, the initial stage (first few days) is followed usually by a steady increase in urine output, which, however, may not return to normal for one to two weeks (period of oliguria). The urine specific gravity is subnormal (hyposthenuria) and low values are obtained for urea, inulin, and PAH clearances, and for PSP excretion. Azotemia persists, but may diminish somewhat. The persistence of these abnormalities is due apparently to continued back-diffusion of excessive amounts of the modified glomerular filtrate and persisting diminution in renal blood flow despite restoration of normal blood pressure and volume (interstitial edema?). It is important to appreciate the fact that there may be no initial period of severe oliguria in certain cases of ischemic necrosis ("lower nephron nephrosis"). Azotemia may occur with urine volumes as large as 1000 cc. daily (low specific gravity), especially in diabetic coma, protracted severe diarrhea, and alkalosis due to injudicious alkali therapy in patients with peptic ulcer.

In the majority of cases, the period of oliguria is followed by one of diuresis, with rather rapid restoration of normal values for urea, inulin, and PAH clearances, and disappearance of azotemia. Hyposthenuria and decreased PSP excretion usually persist for some time after the urea clearance has returned to normal. This is probably attributable to functional immaturity of the newly regenerated tubular epithelial cells, which accounts also perhaps for the diuresis.

Pyelonephritis. In acute pyelonephritis, the renal lesion involves primarily the interstitial kidney tissue, mainly in the medulla, and there is usually no associated demonstrable abnormality of renal function, except in fulminating cases, in which diminished glomerular filtration and renal blood flow may result from severe interstitial inflammatory edema (pressure). If the process becomes chronic, functional renal tissues (glomeruli and tubules) may be destroyed in the areas of involvement and be replaced by fibrous tissue. In this stage, too, all renal functions may be well preserved because of the patchy distribution of the inflammatory lesions. There is some evidence that during the progress of the condition, tubular function (PAH clearance and Tm) undergoes proportionately greater reduction than does glomerular filtration (inulin and urea clearances).

When destruction of or damage to nephrons progresses to the point where the residual functioning elements are unable to maintain normal kidney function, the usual function tests yield essentially the same findings as in chronic glomerulonephritis, except that nephrotic manifestations (p. 618) are extremely unusual (i.e., severe hypoproteinemia, hypercholesterolemia, edema). PSP excretion and concentrating ability may be affected earlier and to a greater degree than urea clearance, until terminal stages. Other evidences of tubular functional impairment in chronic pyelonephritis are discussed elsewhere, e.g., renal tubular acidosis (p. 337), and osteomalacia (p. 623).

Benign Nephrosclerosis (Essential Hypertension). The characteristic findings with clearance procedures in benign nephrosclerosis are indicated in Table 41 (p. 585). In the great majority of cases, until rather late in the course of the disease, glomerular filtration is well maintained (i.e., inulin and urea clearances fairly normal), despite a reduction in renal blood flow (PAH clearance). This results in an increased filtration fraction, implying disproportionate constriction of efferent glomerular arterioles. The renal ischemia also implied by these findings is usually reversible for a rather long period in the course of this disease, as indicated by the response to therapeutic measures designed to relieve arteriolar spasm, e.g., pyrogens, sympathectomy, vasodilator drugs. During this period, i.e., before the development of extensive, irreversible glomerular damage (arteriolosclerosis), all of the usual renal function tests may yield essentially normal results (i.e., urea clearance, PSP excretion, concentration test) in the absence of complications, e.g., congestive heart failure due to hypertension, or acute left ventricular failure due to the same cause or to coronary occlusion.

As the disease progresses, all function tests tend to become abnormal, although advanced renal failure, with azotemia, is unusual in uncomplicated cases. The patient usually succumbs to some consequence of hypertension, e.g., heart failure, coronary occlusion, cerebral vascular disease, before the kidney lesion progresses to this point. This statement does not apply to the rapidly progressive condition referred to as "malignant" nephrosclerosis.

Malignant Nephrosclerosis (Necrotizing Arteriolitis). In this condition, the functional findings are essentially identical with those obtained in rapidly progressing, severe chronic glomerulonephritis. There is steady and rapid deterioration of urea clearance, PSP excretion, and concentrating ability, and increasing blood NPN retention.

"Lipoid Nephrosis." There is still some difference of opinion as to whether so-called "lipoid nephrosis" is a disease entity and not a peculiar manifestation of glomerulonephritis. It is generally agreed that if it does constitute a disease entity, it probably occurs only in children; virtually all adults (and some children) presenting this syndrome eventually exhibit evidence of glomerular damage (hematuria, impaired renal function, hypertension), and develop the clinical picture of chronic glomerulonephritis.

The characteristic biochemical features of this condition include: oliguria (high specific gravity); marked albuminuria (p. 176) (with hyalin, granular and fatty casts, but not hematuria); hypoproteinemia, with extensive edema (p. 156); hypercholesterolemia (hyperlipemia) (p. 115); frequently, hypocalcemia (nondiffusible fraction) (p. 238); hypometabolism (decreased BMR, p. 376). These striking features occur in the absence of demonstrable evidence of renal functional impairment. There is no abnormality of PSP excretion, concentrating ability, or acid-base balance (normal ammonia formation and urine acidification), despite the presence of large amounts of albumin and large numbers of casts (including granular casts) in the urine. High urea clearance values (up to 200% of the average normal) may be obtained, attributed by some mainly to increased glomerular permeability and filtration (increased inulin clearance) and by others mainly to increased renal blood flow (increased PAH clearance). The blood nonprotein nitrogenous constituents are within normal limits. Differentiation of this condition from chronic glomerulonephritis (nephrotic stage) is based largely on the absence of evidence of renal functional impairment and of glomerular damage (hematuria, hypertension).

Toxemias of Pregnancy. Albuminuria may occur in the pernicious vomiting of pregnancy and preeclamptic and eclamptic toxemias. It is usually most marked in eclampsia, but may, in rare instances, be absent in that condition. Some degree of edema, latent or frank, is almost invariably present in true toxemia of pregnancy. This has been shown to be associated with a decrease in the plasma protein concentration. This decrease occurs practically entirely in the albumin fraction and is more marked in eclampsia than in the nonconvulsive forms of pregnancy toxemias. As in the case of normal pregnancy, this decrease in plasma protein concentration is aggravated by deficient protein intake. Although the degree of hypoproteinemia may not in itself be sufficient to produce edema, it favors the development of this phenomenon in the presence of other contributing factors.

In mild and moderately severe cases of pernicious vomiting of pregnancy, the nonprotein nitrogenous constituents of the blood remain within normal limits. In severe cases, however, they may be increased. This increase may be associated with a state of dehydration and electrolyte depletion which is at times dependent upon excessive and prolonged vomiting (p. 591). The blood amino acids may be simultaneously increased. In preeclampsia and eclampsia the total NPN and urea N are usually within normal limits. Most observers report an increase in blood uric acid, and uric acid clearance (p. 201) may be decreased. This is frequently the only demonstrable abnormality in blood nonprotein nitrogenous constituents. In occasional cases with urinary suppression the nonprotein nitrogenous constituents of the blood may be considerably increased. Similar increases are also observed in (*a*) nephritis complicating pregnancy, which may be confused with eclampsia, (*b*) renal cortical necrosis, which at times occurs during or immediately following pregnancy, and (*c*) occasionally in severe hepatic necrosis with an associated "hepatorenal syndrome" (p. 610).

The hypertensive toxemias of pregnancy are commonly regarded as manifestations of a generalized vascular reaction eventuating in chronic vascular disease in about 25 per cent of cases. The special predilection of pregnant women for the development of these manifestations may be dependent, in part at least, upon certain physiologic changes which occur during this period. Among these are physiologic hydronephrosis, which predisposes to pyelonephritis and prevents proper drainage and elimination of infection, and the physiologic hypoproteinemia, which enhances the tendency to edema. A slight temporary increase in renal blood flow has been observed during pregnancy in women with essential hypertension, with no change in glomerular filtration and tubular excretory mass. The blood urea clearance is usually within normal limits in eclampsia. It has been found, however, that reexamination of such cases about three months postpartum reveals a significant decrease in blood urea clearance in 25 to 50 per cent of cases, suggesting the presence of permanent renal or renal vascular damage. A decrease in glomerular filtration rate and an increase in renal blood flow have been observed in toxemia of pregnancy. The renal blood flow returned to norm following delivery in about one-half the cases and fell to ischemic levels in t remainder, which developed persistent hypertension. Renal function tests are particular value in distinguishing between true eclampsia and glomerulonephriti complicating pregnancy.

Congestive Heart Failure. In congestive heart failure, the urine is chara teristically of small volume, and contains albumin, casts, and red blood cel The urine specific gravity is usually elevated in proportion to the diminution volume, in the absence of complicating kidney damage. Urea clearance and PS excretion are usually decreased; diminution in glomerular filtration is indicat by low inulin clearance, and a relatively smaller reduction in renal blood flow decreased PAH clearance. As indicated elsewhere (p. 586), these chang probably result from decreased cardiac output and increased intrarenal arterio constriction (as in shock).

OTHER BIOCHEMICAL MANIFESTATIONS OF RENAL INSUFFICIENCY

As might be anticipated in view of the fundamental role of the kidney in t preservation of the normal composition of the body fluids, renal functio insufficiency results in widespread metabolic disturbances. Most of these a indicated elsewhere in discussions of various aberrations of metabolism (prote lipid, water, electrolyte, acid-base balance, etc.), and it will therefore suff here merely to review briefly the more important of these abnormalities.

Na and Cl Depletion; Dehydration. The progression of chronic glom ulonephritis is accompanied by increasing loss of Na, Cl, and water, eventua in severe dehydration in the terminal stage of the disease. This is due basically urinary excretion of excessive amounts of Na, and approximately correspond amounts of Cl and water, as a result of progressively diminishing tubular capac for urine acidification and ammonia formation, with consequent decrease in reabsorption (p. 330). This condition is also frequently contributed to by vom ing and diarrhea, which occur commonly in patients with advanced renal sufficiency (uremia).

In the majority of cases, the state of depletion of Na and Cl may be masked the simultaneous water loss, and the serum Na and Cl may remain within norm limits (p. 283). However, in certain cases, especially in advanced stages, electrolyte loss is disproportionately great, and hyponatremia and hypochlore (especially with vomiting) develop. Cases of glomerulonephritis are encounte occasionally that present a syndrome resembling adrenocortical insufficie (Addison's disease); namely, hyponatremia, hypochloremia, hypotension, collapse.

Hyperchloremia is observed occasionally. This is unusual in advanced glom ulonephritis except when excessive quantities of NaCl are administered. It n occur also occasionally in such cases during periods of rapid mobilization large amounts of edema fluid.

Serum K Abnormalities. As in dehydration due to other causes, that

nic renal insufficiency, e.g., in chronic glomerulonephritis, produces deple-
of body potassium (p. 281). Usually, the serum K concentration remains
n normal limits. It may fall during administration of K-free fluids (p. 288).
he other hand, it may rise sharply to dangerous levels during injudicious
nistration, especially if parenteral, of potassium salts, due to inadequate
excretion. Hyperpotassemia occurs spontaneously at times in the course of
e renal insufficiency, acute or chronic, accompanied by excessive cell
uction or protein catabolism (severe anoxia, dehydration, fever, necrosis,
uration, etc.), with consequent mobilization of intracellular K faster than it
be eliminated by the damaged kidneys.

ocalcemia; Hypercalciuria; Osteomalacia. Some degree of hypo-
emia occurs commonly in advanced renal failure. The decrease in serum
um concentration is due to hypoproteinemia (p. 238) or hyperphosphatemia
42), or to both. In the case of the former, the decrease is in the nondiffusible
ion; in the latter case it is in the diffusible fraction, and may result in tetany
239).

he occurrence of skeletal lesions (renal osteodystrophy, renal hyperparathy-
sm, renal rickets) in subjects with progressive renal insufficiency is dis-
ed elsewhere (p. 234). In such cases the serum phosphate is elevated, and
serum calcium concentration tends to be low. Occasionally, patients with
onic pyelonephritis present manifestations of osteomalacia, with excessive
ary calcium and phosphate excretion, urinary calculi, normal serum Ca
centration, and increase in alkaline phosphatase activity. This clinical picture
embles portions of the Fanconi syndrome (p. 244). It has been attributed, in
onic pyelonephritis, to the predominantly tubular lesion (p. 619), which
ults in inadequate reabsorption of Ca and other cations, due to decreased urine
dification and ammonia formation, and of PO_4; these substances are therefore
in excess because of the disproportionately large volume of glomerular
rate.

idosis. Acidosis of the "metabolic" type (primary reduction in bicarbo-
e) develops almost inevitably as renal insufficiency progresses. It is an invari-
e feature of advanced renal failure, and of inadequate excretion of urine due
other causes, e.g., urinary obstruction. This is a reflection of inadequacy of the
portant function of the kidneys in the maintenance of acid-base equilibrium.
s characterized by a decrease in the concentrations of sodium (p. 284) and
arbonate (p. 337) in the blood plasma. The methods of study and characteristic
ings are discussed in the section on acid-base balance (p. 337), and it will
ice here merely to review the factors involved in the pathogenesis of acidosis
enal insufficiency.

ECREASED AMMONIA FORMATION AND URINE ACIDIFICATION. Under normal
ditions, cells in the distal segment of the tubule form H ions (from H_2CO_3)
330) and NH_3 (from glutamine and amino acids) (p. 331), which are passed
the lumen of the tubule in exchange for Na ions; the latter are reabsorbed,
HCO_3 ions (produced within the cells from H_2CO_3), into the blood plasma.
mechanism ($Na^+ : H^+$ exchange) plays an essential role in the maintenance

of the normal concentrations of Na and HCO_3 in the extracellular fluids (p. 330)

These functions may be impaired in diseases of the kidney in which the tubular epithelium is damaged or in which a large portion of the functioning renal tissue is destroyed, e.g., glomerulonephritis, "tubular nephritis" ("tubular acidosis") (p. 337), chronic pyelonephritis, malignant nephrosclerosis, polycystic disease, and other destructive lesions (tuberculosis, malignancy). Under these circumstances, the titratable acidity and ammonia content of the urine are decreased, in contrast to the increase (compensatory) which occurs in these factors in acidosis due to other causes (p. 339).

Retention of "Acid Radicals" (Anions). The serum inorganic phosphate is increased in some cases of renal insufficiency also exhibiting an increase in blood NPN or urea N (p. 242). It is probably due usually to decreased glomerular filtration or, in cases of necrotizing nephrosis, in part at least, to excessive back-diffusion. In most cases the degree of hyperphosphatemia roughly parallels the increase in blood creatinine. Values as high as 24 mg./100 cc. have been observed in chronic glomerulonephritis, concentrations of 10 mg./100 cc. in this condition (in adults) usually indicating an early fatal termination. The serum Ca concentration tends to fall as a result of this increase in serum PO_4 (p. 239).

Abnormal retention of phosphate in the extracellular fluids, displacing and thereby lowering bicarbonate, is a factor in the production of acidosis in renal failure or urinary obstruction. Whereas acidosis may occur in renal disease in the absence of hyperphosphatemia, it is almost invariably present when phosphate retention exists.

What has been said concerning phosphate holds true also for sulfate (p. 263). Inasmuch as the urine is the main channel of elimination of this ion also, its concentration in the blood plasma may increase in renal insufficiency, usually of advanced degree (i.e., urea N retention). Values above 5 mg./100 cc. have been reported, but are rare.

The "alkali" (HCO_3^-) deficit in renal failure is usually accountable for largely by the simultaneous decrease in total "base" (mainly Na) (p. 284) and increase in phosphate and sulfate. In some cases, however, a considerable fraction of the plasma anion increment consists of organic radicals, the exact nature of which is not known. They may include amino acids, keto-acids, etc.

Chloride Retention. As indicated elsewhere (p. 284), the dehydration which occurs in association with renal insufficiency results in depletion of body chloride, and, frequently, in hypochloremia. If vomiting is excessive, large amounts of free HCl may be lost by this route, which may, in some measure, offset the tendency toward acidosis. Rarely, it may result in actual alkalosis; a blood pH of 7.6 has been reported under such circumstances. However, this does not occur frequently because the vomitus in patients with uremia usually contains but little HCl.

Parenteral administration of NaCl solution to subjects with impaired kidney function may result in retention of abnormal amounts of Cl^-, and displacement of equivalent amounts of HCO_3^- from the extracellular fluids, aggravating the HCO_3^- deficit ("chloride acidosis"). This is due to the fact that correction of the existing abnormality in acid-base balance requires a larger increment in Na than in Cl in the extracellular fluids. When NaCl solutions are given, the two ions are provided in equal amounts, and the functionally impaired kidneys cannot effectively perform their normal function of excreting the superfluous Cl ions.

Hypoproteinemia. Changes in plasma proteins in glomerulonephritis and nephrosis are discussed elsewhere in detail (p. 156 ff.). They are characterized by variable decrease in albumin (p. 156) and increase in globulins, mainly γ-globulin (p. 162), the former usually predominating, with consequent hypoproteinemia. These changes, although they may occur at any stage of glomerulonephritis, are most pronounced in the nephrotic (chronic active) stage and also in "lipoid" nephrosis.

In advancing renal insufficiency, especially in the terminal stage of chronic glomerulonephritis, the plasma albumin (and total protein) concentration, if previously low, tends to increase, and may return to normal. This is due largely perhaps to the associated dehydration and hemoconcentration. It may be dependent in part upon the diminished elimination of albumin in the urine which not infrequently occurs as the chronic active stage of glomerulonephritis progresses into the terminal stage of uremia. This is due presumably to the complete occlusion of increasingly large numbers of glomeruli, with consequent diminution in the filtration surface area which previously, while blood was able to flow through the inflamed glomerular capillaries, allowed the passage of relatively large quantities of albumin into the glomerular filtrate. This diminution in albuminuria is not observed in all cases.

Magnesium. Although some observers have reported an increase in the concentration of magnesium in the serum in uremia, normal values are obtained in the great majority of cases. There is some evidence that the administration of magnesium salts to patients with renal functional impairment of advanced degree may significantly increase the level of serum magnesium and may result in manifestations of depression of the central nervous system.

Plasma Cholesterol. The plasma cholesterol concentration varies considerably in patients with chronic glomerulonephritis and other forms of renal disease. The acute and chronic active stages of glomerulonephritis are not infrequently accompanied by an increase in the plasma cholesterol concentration. In the terminal stage, however, the development of uremia is not infrequently accompanied by a decrease in plasma cholesterol which may fall to exceedingly low levels (50–60 mg. per 100 cc.). At the same time the total fat, fatty acid and phospholipid concentrations in the plasma may be increased. A similar lowering of the plasma cholesterol concentration may occur in uremia due to other causes. It appears that in chronic glomerulonephritis a decrease in plasma cholesterol from a previously elevated or normal level is of serious prognostic significance, especially if associated with increasing nitrogen retention.

Many believe that the hypocholesterolemia of uremia is probably due in large measure to contributing factors such as anemia, starvation and cachexia. As is well known, these factors may in themselves produce, or at least be accompanied by a fall in plasma cholesterol. It has been found, however, that hypocholesterolemia of severe degree occurs frequently in terminal states unassociated with nitrogen retention or renal disease, in many of which anemia, cachexia and starvation can be excluded from consideration as important etiologic factors. Among these are coronary artery occlusion, congestive and left ventricular heart failure, peritonitis, pneumonia and certain cases of urinary obstruction due to prostatic enlargement.

Phenol and Other Organic Substances. It has been shown that uremia is usually accompanied by increased concentrations of phenols and related substances in the body fluids. The interpretation of this observation is rendered difficult by the nonspecificity of the methods employed for the determination of phenols. Phenol, paracresol, indole and other related substances are formed in the body as a result of processes involving deamination, decarboxylation and oxidation of the aromatic amino acids, tyrosine, phenylalanine and tryptophan. These phenols appear to be formed chiefly, if not entirely, as a result of bacterial action on protein derivatives in the intestines. Under normal conditions, these substances are absorbed from the gastrointestinal tract and are detoxified by conjugation with sulfuric or glycuronic acids. The adequacy of this detoxification depends upon the functional integrity of the liver, intestines and other organs that may be involved in these processes. It has been suggested that certain of the manifestations of the uremic syndrome are dependent upon the accumulation in the blood and other body fluids of phenols and other aromatic compounds. Some observers attribute considerable significance to high values for indican in the blood serum as an indication of approaching uremia. Concentrations of 5 to 10 mg./100 cc. are not unusual in renal failure (normal 0.05–0.15 mg.).

As is the case with phenol, the evaluation of the significance of guanidine studies is complicated by the lack of specificity of the methods employed for the determination of this substance. However, several investigators have reported an increase in the "guanidine" content of the blood in patients with uremia and have attributed to this increase some of the toxic manifestations of the uremic state.

A modification of the van den Bergh reaction (p. 518) is useful occasionally in rapidly differentiating uremic coma from coma due to other causes. The test (diazo test) is performed as follows:

To 1 cc. of blood plasma or serum add 2 cc. of 95 per cent alcohol. To 1 cc. of the supernatant fluid obtained following centrifugation add 0.25 cc. of the van den Bergh reagent and boil gently for thirty seconds. In the serum of normal individuals there is either no color change or the development of a faint pink color (bilirubin). A positive uremic reaction consists in the appearance of an orange-buff color. Add a few drops of 40 per cent NaOH. In the case of normal individuals (nonuremic) a green color appears; a positive uremic reaction consists in the appearance of a definite pink or cherry-red color which lasts from a few seconds to several hours, depending upon the concentration of the responsible agent.

In the great majority of instances the positive reaction is only obtained in the presence of advanced renal insufficiency, as evidenced by marked retention of nonprotein nitrogen and acidosis. On the other hand, cases have been occasionally reported in which a positive reaction is obtained before significant nitrogen retention has occurred. The nature of the substance responsible for this reaction has not been established with certainty. Alcoholic extracts of normal urine give the same reaction, and it is believed by some that the responsible substance is an indoxyl compound, probably indican, or possibly, in part, indoxyl glycuronate. A positive reaction is of value chiefly from the standpoint of prognosis, usually indicating a rapidly fatal outcome. This test cannot be applied in the presence of hyperbilirubinemia, which causes the development of a green or violet color following the addition of NaOH, which will obscure the light cherry-red uremic reaction.

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CHAPTER XXIII

Cerebrospinal Fluid

PROTEIN

The protein content of cerebrospinal fluid is lower than that of any other normal body fluid with the exception of the aqueous humor of the eye, which closely resembles the cerebrospinal fluid in chemical composition. The amount of protein in normal fluid obtained by lumbar puncture is 15 to 45 mg. per 100 cc., cisternal fluid containing 10 to 25 mg. per 100 cc. The appearance of increased amounts of protein in the fluid is dependent upon the same factors which govern the presence of protein in all body fluids, normal and pathologic. Chief among these is the permeability of the barrier interposed between the plasma and the subarachnoid space, which, under normal circumstances, may be considered to be the choroid plexuses. Normally these vessels, as well as those of the meninges, are practically impermeable to proteins; in inflammatory conditions of the brain, cord and meninges the capillary walls become more permeable, and, depending upon the degree of inflammation, increasing amounts of albumin, pseudoglobulin, euglobulin and fibrinogen pass into the subarachnoid space, the last two appearing only in severe inflammatory processes.

Increase in the protein content may be found in conditions, other than inflammations, in which capillary and cell permeability are increased. These include: toxic states such as uremia, pneumonia and typhoid fever; convulsive states, as epilepsy and spasmophilia; conditions causing generalized or localized elevation of subarachnoid tension, as brain and spinal cord tumor.

Since in most pathologic fluids containing an increased quantity of protein globulin is present in abnormally high concentration, globulin tests are commonly used as a means of roughly estimating the protein content of cerebrospinal fluid. The most popular of these tests are the Noguchi, Nonne-Apelt, Ross-Jones and Pandy reactions; the results are reported as normal, 1 plus, 2 plus, 3 plus and 4 plus, depending upon the amount of globulin precipitated. More exact quantitative determinations may be made, the results being recorded in milligrams per 100 cc. of fluid.

Meningitis. Inflammatory exudates in any situation contain comparatively large amounts of protein. In the various forms of suppurative meningitis the total protein content of the cerebrospinal fluid is high (125–3000 mg. per 100 cc.), consisting of globulin, albumin and, at times, small amounts of fibrinogen. High values are also the rule in tuberculous meningitis (200–2000 mg.) and in acute luetic meningitis (180–540 mg.). In true meningitis the increase in protein usually parallels the increase in cellular content. It must be realized that in some cases of early meningitis the fluid protein may be normal. According to some observers, the determination of qualitative differences in the cerebrospinal fluid protein may be of value in distinguishing between tuberculous and other forms of meningitis. The Levinson test consists essentially in the comparison of the amount of precipitation produced in the cerebrospinal fluid by mercuric chloride and sulfosalicylic acid. A precipitate volume ratio of 2 to 1 or higher (bichloride precipitate: sulfosalicylic acid precipitate) is regarded as highly suggestive of tuberculous meningitis under the conditions of the test. The general experience is that this test is of only corroborative value and that it has little if any specific diagnostic significance.

Serous Meningitis. This term is applied to a condition of meningeal irritation of a noninfective nature which may be due to toxic states such as pneumonia, influenza, typhoid fever, and the like, or uremia, or to inflammation of structures adjacent to the cranial cavity, i.e., otitis media and mastoiditis.

The cerebrospinal fluid in serous meningitis or meningismus is, in most cases, entirely negative except for some increase in amount and pressure. However, in isolated cases, abnormally high concentrations of protein may be found (18–70 mg.), other chemical and cytologic findings being normal. This protein increase may be comparatively slight and not detectable by the routine procedures for globulin determination.

Table 43. Protein in Cerebrospinal Fluid

CONDITION	SPINAL FLUID*	CISTERNAL FLUID*
Normal	15– 45	
Tabes	40– 200	
Paresis	30– 190	
Cerebrospinal lues	20– 160	
Pneumococcic meningitis	150–2000	
Meningococcic meningitis	180–3000	
Streptococcic meningitis	160–2000	
Tuberculous meningitis	200–2000	
Brain tumor	30– 500	
Brain abscess	30– 500	
Froin syndrome	80–3000	25–180
Epidemic encephalitis	50– 300	
Cerebral hemorrhage	20– 220	
Cerebral thrombosis	20– 140	
Epilepsy	15– 80	
Serous meningitis	20– 70	
Myxedema	60– 200	

* Expressed in milligrams per 100 cc.

Convulsive States. During and shortly after epileptic seizures there may be an increase in cerebrospinal fluid protein. Several investigators have reported increased albumin in the fluid in this condition. In the interval between convulsions the fluid is essentially normal. The same is true of the convulsions of spasmophilia of children. The protein concentration rises also during uremic convulsions.

Organic Disease of Brain and Cord. Protein is increased in many organic diseases of the brain and cord, with or without associated pathologic change in the meninges. In the luetic affections, paresis, tabes and cerebrospinal syphilis, values of 20 to 200 mg. per 100 cc. are found, usually accompanied by an increase in cells. In epidemic encephalitis the protein ranges from 50 to 300 mg. per 100 cc. In cerebral hemorrhage, thrombosis and embolism the protein content may be normal or increased (20–220 mg.). In brain abscess without meningitis and in brain tumor with increased intracranial pressure the protein varies from 30 to 100 mg. per 100 cc. with slight or no cellular increase. The highest values for protein are found in fluids presenting the so-called "Froin syndrome" (xanthochromia, greatly increased protein, spontaneous coagulation and mononuclear pleocytosis). This syndrome is characteristic of spinal cord compression, due usually to tumor, associated with retention of spinal fluid in a cul-de-sac. Values of over 2000 mg. per 100 cc. have been reported in this condition. The protein content of the fluid above and below the point of obstruction may show marked differences in protein concentration. In one series of cases the protein content of the fluid removed by cisternal puncture was 24 to 180 mg. per 100 cc. while that obtained by lumbar puncture contained 80 to 3000 mg. per 100 cc. Such findings are of considerable diagnostic importance.

Myxedema. The protein content of cerebrospinal fluid is rather consistently increased in myxedema. Values as high as 100 mg. per 100 cc. are observed commonly and up to 200 mg. occasionally.

GLUCOSE

The concentration of glucose in the cerebrospinal fluid is dependent upon: (1) the blood sugar concentration; (2) the permeability of the protective barrier, represented by the lining of the choroid plexuses and possibly of the capillary terminations of the cerebrospinal vessels which are surrounded by prolongations of the subarachnoid space; (3) the rate of glycolysis within the fluid. It has been fairly well established that, in the absence of disease of the brain, cord or meninges, the concentration of sugar in the cerebrospinal fluid is approximately 60 to 70 per cent of that in the blood within a wide range of values extending from moderate hypoglycemia (40 mg.) to moderate hyperglycemia (400 mg.). The normal range has been found to be from 40 to 70 mg. per 100 cc. in the fasting adult. The normal values for children up to ten years of age have been found to be 70 to 90 mg. per 100 cc. It has been shown that the fluid must be withdrawn under fasting conditions, and, in order to be properly interpreted, must be compared with the sugar content of the blood withdrawn at the same time. There is a delayed postprandial rise in spinal fluid sugar following that which occurs in the blood. Because of the lack of sufficient information regarding the blood and cerebrospinal sugar curve relationship following the ingestion of food, no significance should be attached to variations in the ratio of spinal fluid sugar to blood sugar except in the fasting state. The sugar content of ventricular and cisternal fluid is usually slightly higher than that of lumbar spinal fluid.

Practically all injuries to semipermeable membranes result in an increase in their permeability. Hence, it is naturally inferred that, as with protein, any disease associated with injury to the cerebrospinal vascular system, particularly the choroid plexuses and cerebrospinal capillaries, may result in an increase in the sugar content of the spinal fluid, a condition termed hyperglycorrhachia. A decrease in spinal fluid sugar, hypoglycorrhachia, is almost invariably dependent upon an increased rate of glycolysis, this being probably the only agency by which the sugar concentration can be lowered independently of a diminution in the sugar content of the blood. Increased glycolysis occurs most strikingly in the suppurative meningitides, and to a lesser degree in tuberculous meningitis and in some cases of luetic meningitis. It is due to the glycolytic action of either the leukocytes or the organisms present in the fluid.

Hyperglycorrhachia. Epidemic Encephalitis. A frequent finding in the cerebrospinal fluid of patients with acute epidemic encephalitis is an increased glucose concentration. The figures range from 70 to 110 mg. per 100 cc. in adults. The presence of a subnormal spinal fluid sugar concentration militates against the diagnosis of acute epidemic encephalitis, a fact of importance in view of the varied symptomatology of this condition and the difficulty occasionally encountered in differentiating it from other disorders, particularly tuberculous meningitis.

Table 44. Glucose in Cerebrospinal Fluid

CONDITION	SUGAR*
Normal fasting adult	40– 70
Normal fasting child (to ten years)	70– 90
Functional mental disease	70– 95
Lues of central nervous system	30–110
Epidemic encephalitis	70–110
Suppurative meningitis	0– 25
Tuberculous meningitis	18– 36
Brain abscess	70–110
Brain tumor	70–110

* Expressed in milligrams per 100 cc.

Syphilis of the Central Nervous System. Increased sugar concentrations are found in some cases of syphilis, chiefly in that form affecting the cerebrospinal vessels particularly, the vascular form of cerebrospinal syphilis with little or no meningeal involvement. The figures range from 70 to 110 mg. per 100 cc. In cerebrospinal syphilis of the parenchymatous type, or paresis, with slight meningeal lesion, the sugar content is normal, as it is in most cases of tabes. In acute luetic meningitis, low normal or slightly subnormal values are obtained.

Increased Intracranial Pressure. High sugar values (70–110 mg.) are usually found in conditions causing marked increase in intracranial tension. These include brain tumor, convulsive disorders, and brain abscess not associated with meningitis. Increased sugar concentrations are present in some cases of serous meningitis, particularly in that type associated with uremia.

Functional Mental Disorders. Certain psychiatric disorders, particularly dementia praecox, may be accompanied by hyperglycorrhachia (70–95 mg.). This finding is not constant and is of no diagnostic value in these conditions.

Diabetes Mellitus. As stated above, high spinal fluid sugar values may be due to hyperglycemia. Recognition of this fact is essential for the proper interpretation of spinal fluid sugar determinations.

Hypoglycorrhachia. Suppurative Meningitis. In all cases of acute meningitis associated with polymorphonuclear pleocytosis, sugar is either entirely absent or present only in very small amounts (0–25 mg. per 100 cc.). Improvement in the condition of patients with meningococcic meningitis following the administration of antimeningococcic serum is accompanied by a rise in the spinal fluid sugar concentration.

Tuberculous Meningitis. Hypoglycorrhachia is an invariable feature of the fluid of tuberculous meningitis (18–36 mg. per 100 cc.). Sugar is rarely entirely absent, as in the suppurative meningitides. This finding is of importance from the standpoint of differential diagnosis, particularly, as stated above, from acute epidemic encephalitis, which may in other respects simulate it very closely.

Luetic Meningitis. In a comparatively small number of cases of syphilis of the central nervous system, particularly those involving primarily the meninges and associated with a marked pleocytosis, the sugar content of the fluid may be slightly decreased (30–40 mg. per 100 cc.).

NONPROTEIN NITROGENOUS CONSTITUENTS

Practically all of the known nonprotein nitrogenous constituents of the blood plasma are represented in the cerebrospinal fluid. The concentration of these substances in the fluid differs from that in the blood, however, depending upon their degree of diffusibility. The total nonprotein nitrogen varies from 12.5 to 30 mg. per 100 cc. Urea, being one of the most diffusible constituents of the blood, exists in all body fluids in practically the same concentration. The urea content of cerebrospinal fluid, expressed as nitrogen, is 6 to 15 mg. per 100 cc. The creatinine varies between 0.45 and 1.5 mg., amino acids from 1 to 4 mg., and uric acid from 0.25 to 1.0 mg. per 100 cc., the last being slightly higher in children (0.3–1.5 mg.). The residual or undetermined nitrogen normally is approximately 50 per cent of that of the blood, ranging from 2 to 6 mg. per 100 cc.

The determination of these constituents of the spinal fluid is in most instances of little practical benefit, either diagnostically or prognostically. With the possible exception of uric acid, they are altered in few conditions other than nephritis and uremia. Uric acid has been reported in increased concentration in all forms of meningitis. In most cases of nephritis and uremia the ratio between the total nonprotein nitrogen of the spinal fluid and blood is maintained, values as high as 375 mg. per 100 cc. having been reported. As these values increase, the urea nitrogen fraction constitutes an increasingly large proportion of the total. Thus, in one case, with a total nonprotein nitrogen of 110 mg. per 100 cc., the urea nitrogen was found to be 90 mg. per 100 cc. Creatinine, uric acid and amino acids usually increase in proportion to their concentration in the blood. In some cases of uremia, strangely enough in the asthenic type, without convulsions, the undetermined nitrogen fraction is found to be increased out of all proportion to its level in the blood. In a few instances it constituted 70 to 75 per cent of the total nonprotein nitrogen, which ranged between 240 and 375 mg. per 100 cc.

As stated above, at the present time little or no practical importance can be attached to the determination of these constituents of the spinal fluid. However, the findings in nephritis and uremia are of considerable interest in view of the insight which they afford into the concentration of the various nonprotein nitrogen fractions in the tissues and body fluids as contrasted with that in the blood.

CHLORIDE

The chloride content of the cerebrospinal fluid is from 720 to 750 mg. per 100 cc., expressed as sodium chloride. Values in infants exhibit slightly more variation, ranging from 650 to 750 mg. per 100 cc. This increased concentration in the spinal fluid as compared with that in the plasma (570–620 mg.) is explained in part on the basis of the Donnan equilibrium governing the concentrations of ions on either side of a semipermeable membrane (the capillary walls of the choroid plexuses) when the fluid on one side (plasma) contains molecules which are not diffusible (protein) (see p. 306). The spinal fluid chloride has been found to be increased in some cases of nephritis, but is of neither diagnostic nor prognostic importance in that condition.

In most forms of meningitis the chloride is decreased, the diminution being most marked in tuberculous meningitis. In the suppurative meningitides the values range between 600 and 700 mg. per 100 cc. In tuberculous meningitis the figures are quite consistently below 600 mg., usually being from 450 to 580 mg. per 100 cc. In luetic infections of the central nervous system, in epidemic encephalitis, poliomyelitis, and in practically all other diseases of the brain and cord the chloride content of the cerebrospinal fluid is but little altered. Therefore the determination of chloride is particularly useful in the differential diagnosis of tuberculous meningitis from conditions with which it may be confused, particularly epidemic encephalitis. The decrease in cerebrospinal fluid chloride which occurs in inflammatory conditions of the meninges is probably due in a large measure to the increased protein content of the fluid in those conditions, the more equal distribution of protein between the blood plasma and cerebrospinal fluid resulting in a more equal distribution of chloride in those fluids (see p. 305). The decrease in plasma chloride concentration, which occurs at times in meningitis, also plays an important part in the production of this phenomenon.

Variations in the chloride content of the blood plasma are reflected in corresponding changes in the chloride content of the cerebrospinal fluid. Consequently, in such conditions as lobar pneumonia and upper intestinal or pyloric obstruction, which are commonly associated with hypochloremia, the concentration of chloride in the cerebrospinal fluid is correspondingly decreased. The plasma chloride concentration must therefore be considered in interpreting subnormal values for cerebrospinal fluid chloride, particularly in acute infectious diseases such as pneumonia, in which the development of symptoms of meningeal irritation may arouse suspicion of the presence of meningitis.

INORGANIC PHOSPHATE

The inorganic phosphate content of cerebrospinal fluid averages approximately 30 to 50 per cent of that of blood serum, ranging between 1 and 2 mg. per 100 cc. in adults and from 1.5 to 3.5 mg. in children. It is increased in all forms of meningitis, in nephritis and uremia with phosphate retention, and has been found to be increased in conditions associated with degenerative processes in the brain and cord, such as tumor, tabes and paresis. The determination of the cerebrospinal fluid phosphorus concentration has no practical value.

CHOLESTEROL

Most investigators have found that the normal cerebrospinal fluid contains no cholesterol or minute traces only. An increase occurs in many diseases of the central nervous system, including meningitis (trace–12 mg.), brain tumor and abscess (5–15 mg.), cerebral hemorrhage (5–20 mg.). In the majority of cases of syphilis of the central nervous system the cholesterol content of the fluid is not appreciably increased. In various mental disorders, figures ranging from 0.2 to 0.7 mg. per 100 cc. have been reported.

LACTIC ACID

The cerebrospinal fluid normally contains no lactic acid. If allowed to stand at room temperature, lactic acid is formed in the process of glycolysis of the glucose present in the fluid. In all conditions in which the sugar content of the fluid is decreased, lactic acid will be found to be correspondingly increased. It exists in highest concentration, therefore, in the suppurative meningitides, and, to a lesser extent, in tuberculous meningitis. It has also been found in patients with uremia.

HYDROGEN ION CONCENTRATION

The hydrogen ion concentration of cerebrospinal fluid examined immediately after withdrawal is almost the same as that of the blood, the pH varying normally from 7.4 to 7.6. Levinson has found that upon standing, exposed to air, the alkalinity increases, due to the loss of CO_2 from the fluid. The alkaline reserve, as measured by the CO_2 combining power, is also practically identical with that of blood plasma (55–75 volumes per cent).

The pH of the fluid in tuberculous meningitis is usually within normal limits (7.4–7.6), the alkalinity increasing upon standing, as in normal fluid. In the suppurative meningitides the fluid is slightly more acid (pH 7.2–7.5), the increase being due, in all probability, to the presence of lactic acid. Upon standing, the acidity shows much less tendency to decrease than in the case of normal fluids, actually increasing in some instances (Levinson). This is perhaps due either to an increased rate of glycolysis or fermentation, or to increased production of CO_2 by the cells present in the fluid, balancing the loss of CO_2 which occurs upon standing exposed to air.

SODIUM, POTASSIUM, CALCIUM AND MAGNESIUM

Determination of the sodium and potassium content of cerebrospinal fluid is, as far as is known, of no practical value. Figures for sodium range from 300 to 380 mg. and those for potassium from 16 to 22 mg. per 100 cc., being essentially the same as the values for the concentrations of these elements in the blood plasma under normal and abnormal conditions.

The calcium content of normal cerebrospinal fluid varies from 4.5 to 5.5 mg. per 100 cc. It is increased in practically all cases of meningitis and epidemic encephalitis. This is due to the fact that in the presence of protein in tissue fluids their calcium content is increased by an amount proportional to the quantity of protein present, the added calcium being nondiffusible in nature and the diffusible fraction being essentially unaltered. The terms "diffusible" and "nondiffusible," as here employed, refer to the ability to pass through a semipermeable artificial membrane or the capillary wall. The cerebrospinal fluid calcium is apparently all in ionized form and, under normal conditions, is quantitatively identical with the diffusible fraction of serum calcium. This correspondence between values for cerebrospinal fluid calcium and diffusible calcium of the blood serum as determined by artificial membrane methods is, however, lost under conditions of abnormal serum calcium concentration. For example, marked decrease or increase in serum calcium produced by parathyroidectomy or administration of parathyroid hormone, is accompanied by little or no change in the cerebrospinal fluid calcium concentration. Instances of hypoparathyroidism have been reported in which the serum and spinal fluid calcium concentrations were identical (4.5 mg. per 100 cc.). Low values for spinal fluid calcium may be obtained in patients with renal failure, the decrease being roughly proportional to the increase in spinal fluid phosphate concentration. Some observers believe that there may be some relationship between the cerebrospinal fluid calcium concentration and the manifestations of muscular hyperirritability in uremia, such manifestations occurring frequently with spinal fluid calcium values below 4 mg. per 100 cc.

Reported values for magnesium concentration of the spinal fluid are slightly higher than those for normal blood serum, averaging about 3.3 mg. per 100 cc. It would appear that the spinal fluid magnesium concentration is independent of variations in its concentration in the blood serum. Variations in this element appear to have little practical significance. Increased, normal and decreased values for spinal fluid magnesium have been reported in various forms of meningitis.

XANTHOCHROMIA

Xanthochromia is a term applied to a condition in which the spinal fluid exhibits a clear yellow color. In most cases the pigment responsible for this discoloration responds to the indirect van den Bergh reaction, suggesting its derivation from hemoglobin and its intimate relationship with bilirubin. In some cases in which this reaction is not obtained, the color may be due to some intermediary pigment complex or perhaps to lutein. The intensity of color may be expressed in terms of van den Bergh units or milligrams of bilirubin by the indirect van den Bergh reaction or, more simply, by the estimation of the icterus index.

Xanthochromia may be due to hemorrhage into the subarachnoid space from any cause. If blood is present in the subarachnoid space for more than a few hours, the cerebrospinal fluid, following centrifugation, will exhibit a clear yellow color above the layer of packed red cells. This observation is at times of value in differentiating between pathologic hemorrhage into the subarachnoid space and the introduction of blood into the cerebrospinal fluid by injury to the subdural venous plexus at the time of puncture. The development of the yellow color is due to the occurrence of hemolysis with subsequent transformation of hemoglobin to a pigment closely resembling, if not identical with, bilirubin. If the blood has been present for more than a week the yellow color becomes much more intense and then gradually fades, due probably to reabsorption of pigment, finally disappearing entirely in from one to two weeks, depending upon the extent and duration of the hemorrhage.

In mild and moderately severe icterus, bilirubin, because of its relatively poor diffusibility, does not pass into the cerebrospinal fluid, but in advanced icterus, particularly of the obstructive type, varying amounts of bilirubin may be present in the fluid, imparting a yellow-brown color.

Xanthochromia may occur in compression of the cord by intramedullary tumors or by extradural compression, as in the case of tuberculosis of the vertebra; it is particularly frequently observed in tumors in the region of the cauda equina. Under such circumstances a meningeal pocket is formed and venous stasis occurs with consequent capillary hemorrhage into the cerebrospinal fluid. A similar condition occurs in some cases of acute myelitis and in chronic meningitis with the formation of adhesions. In such cases, particularly in cord tumors, there is, in addition, interruption of the cerebrospinal fluid circulation and transudation of serum in the area of venous and capillary stasis. The cerebrospinal fluid under such circumstances contains large quantities of protein. The term "Froin syndrome" is applied to xanthochromia in a fluid of high protein content which may coagulate spontaneously. This syndrome, because of its frequent occurrence in cases of tumor of the spinal cord, is of considerable diagnostic import.

Xanthochromia of varying degree occurs as a physiologic phenomenon in practically all premature infants during the first two months of life. About 60 per cent of such fluids respond to the indirect van den Bergh reaction, the icterus index being about 1 during the first twenty-four hours of life, increasing to reach a maximum of about 4 during the second week, and then gradually decreasing to disappear in about eight weeks or earlier depending upon the degree of immaturity of the infant. In cases unassociated with hemorrhage into the subarachnoid space, the xanthochromia of premature infants appears to be related to the increased bilirubin content of the blood serum existing during that period (icterus neonatorum). Since bilirubin appears in the cerebrospinal fluid of adults with icterus only when the latter is of severe grade or when there has occurred some damage to the choroid plexus, it appears that the permeability of the blood plasma-cerebrospinal fluid barrier in premature infants and in mature infants during the first few days of life is increased, due perhaps to the incomplete state of development of the cells constituting that barrier.

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